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TITLE: Germline Variation in *HSD3B1* as a Novel Biomarker in Prostate Cancer

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14. ABSTRACT In lay terms, we assessed the clinical impact of inheriting a variant form (allele) of the <i>HSD3B1</i> gene, previously shown to enable prostate cancer cells to make their own derivative of testosterone that can drive disease progression, despite castration. We have demonstrated in three independent groups of men treated with androgen deprivation therapy (ADT) that men possessing the variant <i>HSD3B1</i> allele have worse clinical outcomes (i.e. likelihood of disease progression, metastasis (spread to the bones or other distant sites), and death). Moreover, men who have two copies of the variant allele tend to have profound resistance to ADT. In a fourth cohort of men who received ADT for biochemical failure after initial radiation therapy, we again found that the variant allele predicts for large differences in time to metastasis. Taken together, these results indicate that <i>HSD3B1</i> genotype is a powerful genetic biomarker of resistance to ADT, and can be used to identify men who might benefit from escalated treatment. Identification of these men has potential ramifications for guiding clinicians in augmenting standard ADT with highly-potent inhibitors of the androgen axis or chemotherapy in an effort to improve outcomes. Design and analysis of future studies should take into account <i>HSD3B1</i> genotype, in view of the differences in outcomes according to the number of variant alleles a man inherits.					
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INTRODUCTION

For over seventy years, the most effective and widely used systemic therapy for prostate cancer has been androgen deprivation therapy (ADT). Although nearly all men will respond to ADT, most will develop castration-resistant prostate cancer (CRPC). However, the duration of response to ADT varies widely. Although numerous resistance mechanisms have been described, knowledge of them typically cannot be used to predict *a priori* how well a man is likely to respond to ADT, and therefore cannot be used to guide management. In 2013 we described a novel resistance mechanism resulting from a mutation in *HSD3B1*, the gene encoding 3 β -hydroxysteroid dehydrogenase-1 (3 β HSD1), the enzyme responsible for catalyzing the rate-limiting step in the conversion of adrenal androgen precursors to dihydrotestosterone (DHT), and which is required for all pathways of DHT synthesis. *HSD3B1*(1245A>C) changes amino acid 367N→T and renders 3 β HSD1 resistant to proteasomal degradation, causing profound accumulation and, effectively, gain-of-function. The resultant increased intratumoral metabolic flux of adrenal precursors to more potent androgens, including DHT (the most potent androgen), thus enhances androgen receptor activation and accelerates tumor proliferation, despite castration. Notably, while the *HSD3B1*(1245C) allele can be acquired by mutation, it is also heritable in the form of a single nucleotide polymorphism. Recognizing the possibility for a novel prostate cancer biomarker with the potential to predict resistance to ADT, we hypothesized that men inheriting the *HSD3B1*(1245C) allele would exhibit resistance to ADT. Our preliminary data in a cohort of men with biochemical failure strongly supported this hypothesis, and prompted the current project to validate and expand these findings.

KEYWORDS

Prostate cancer
Androgen deprivation therapy
ADT
Castration-resistant prostate cancer
CRPC
HSD3B1
3 β -hydroxysteroid dehydrogenase-1
3 β HSD1
Resistance mechanisms
Biomarker
Radiation therapy
Dihydrotestosterone
DHT

ACCOMPLISHMENTS

What are the major goals of the project? What was accomplished under these goals?

Major goals include both research and training objectives. The research objectives are discussed here, and the training goals are described in the item below on training opportunities and professional development. For completeness and to provide adequate context for interpretation, this report includes all accomplishments on the project to date. If primarily interested in accomplishments from the reporting period for the current annual report, the reviewer may skip to Specific Aim 3. After our validation work for Specific Aim 1, we proceeded to work on Specific Aim 3 in order to operate most efficiently as data became available.

Specific Aim 1: validate the predictive value of *HSD3B1* genotype in independent cohorts. We have worked extensively on this goal, and published this work in *Lancet Oncology*. Our target time frame was 14 months for the first milestone: validation of the predictive value of *HSD3B1* genotype in an independent cohort using the Mayo Clinic data. We completed this task within the target time frame.

Subtask 1: Define the Mayo Clinic validation cohorts. The post-prostatectomy validation cohort was obtained from the Sponsored Programs of Research Excellence (SPORE) database at Mayo Clinic. We identified all men who: (1) received ADT for biochemical and/or non-metastatic clinical failure following prostatectomy, and (2) had blood samples available for research. To ascertain the impact of *HSD3B1* genotype among men with more advanced disease, we queried a separate Mayo database and identified men enrolled with metastatic CRPC who had blood samples banked for research. To minimize confounding, patients were excluded if they had received radiation therapy (RT) for initial local therapy, since use of ADT with RT was not adequately captured in this cohort. Patients with confirmed or probable neuroendocrine prostate cancer were excluded, given that such cancers are generally not dependent on the AR axis.

*Subtask 2: Obtain germline DNA for the men in the validation cohorts and then determine *HSD3B1* genotype using a high-resolution melting assay developed for this purpose.* We obtained peripheral blood mononuclear cells for genotyping in the Mayo cohorts. Notably, *HSD3B1* has one homolog (*HSD3B2*) and four non-processed pseudogenes, which have very closely related DNA sequences that may obscure detection of the variant sequence. We therefore developed a melting assay using an unlabeled locked nucleic acid oligonucleotide probe in an asymmetric polymerase chain reaction to perform targeted genotyping at this locus.

DNA from the Mayo cohorts was extracted from peripheral blood mononuclear cells. The DNA quantity and quality was measured using NanoDrop (Thermo Scientific, Waltham, MA, USA). Genomic DNA was stored at no more than 4°C. The PCR was performed in 20µL reactions (final volume) using LightScanner Master Mix (Biofire, Salt Lake City, UT, USA), 4µL extracted genomic DNA (10µg/µL), forward primer, reverse primer, and unlabeled LNA probe at concentrations of 0.05, 0.5, and 0.5µM, respectively (Table S1 in the appendix to the first manuscript). The PCR and the melting analyses were performed in StepOnePlus™ System (Life Technologies, Grand Island, NY, USA) using the following conditions: 2 min at 95°C, followed by 60 cycles of 94°C for 30 s, 66°C for 30 s,

and 75°C for 30 s. The amplification cycles were then followed by the melting steps: 95°C for 30 s, followed by cooling to 25°C for 60 s, and a slow final denaturation to 95°C at a thermal ramp rate of 0.3%. Data were analyzed using probe melting derivative peaks. Each validation run was performed with duplicate control samples representing the three different genotypes and each sample was tested in duplicate during the study genotyping. Our melting analysis technique was able to reliably distinguish genotypes (see Figure S1 in the appendix to the first manuscript), and was validated with Sanger sequencing for 60 samples (20 for each genotype) with 100% concordance.

Subtask 3: Extract demographic, treatment, and clinical outcomes data from the clinical annotation in Mayo Clinic's prostate cancer registries. For demographic and treatment factors, please see Table 1 in the first manuscript and Tables S2 and S3 in the appendix to the first manuscript. Note that Table 1 also includes the demographic and treatment factors of the Cleveland Clinic primary cohort, which was the cohort used for the preliminary data guiding this project. Outcomes data on clinical events were likewise extracted—see Subtask 4 for additional information on outcomes.

Subtask 4: Statistical analysis. Progression-free survival, distant metastasis-free survival, and overall survival were analyzed using Kaplan-Meier methods. Log-rank tests were used to compare genotypes. Cox proportional hazards regression was performed to further assess potential allele-dosage effects. Demographic and treatment characteristics were compared across genotypes to assess for confounders using Fisher's exact test and Kruskal-Wallis analysis of variance. Fisher's exact test was utilized rather than the Chi-square test because the latter is less accurate when counts are low. Multivariable Cox proportional hazards models were used to account for potential confounders. All tests were two-sided, and P-values less than or equal to 0.05 were interpreted as statistically significant. Analyses were performed with the use of SAS software, version 9.3 (SAS Institute, Cary, NC). Time-to-event outcomes were measured from the date ADT was originally initiated. The key results are shown in Figures 1-3 in the first manuscript, which demonstrate that progression-free survival, distant metastasis-free survival, and overall survival varied according to *HSD3B1* genotype. More specifically, outcomes worsened as the number of inherited variant alleles increased. Additional details are included in the text of the Results section of the first manuscript and in Tables S2-S4 in the appendix to that manuscript.

Specific Aim 2: Perform an exploratory analysis of serum steroid profiles (delta-4/delta-5 ratios) to evaluate for a biochemical correlate to *HSD3B1* genotype, which could represent an alternate biomarker. Status: to be done.

Specific Aim 3: Determine whether *HSD3B1* genotype remains predictive when ADT is combined with radiotherapy for prostate cancer. For this objective, we identified retrospective and prospective datasets with different strengths. We focused our efforts on analysis of the retrospective dataset first, given that we were able to obtain the necessary data in a timelier fashion. We have recently published our results in *JAMA Oncology*, and have attached the manuscript for reference.

Subtask 4: Analyze a cohort of men treated with radiotherapy for localized prostate cancer and assess the predictive value of HSD3BI genotype. To accomplish this, we used a well-characterized prostate cancer registry to identify men who were treated with ADT for biochemical recurrence after definitive RT. Key clinical data were extracted and systematically quality assured. *HSD3BI* genotype was determined retrospectively from buffy coat by investigators blind to the clinical data using the methodology we developed for Specific Aim 1. Kaplan-Meier methods were used to assess time to progression (TTP), time to metastasis (TTM), and overall survival (OS) from initiation of salvage ADT. We analyzed differences in these endpoints according to genotype using an additive genetic model with the log-rank trend test. Progression was defined as the first occurrence of: (1) prostate-specific antigen (PSA) progression; (2) radiographic progression; or (3) initiation of second-line therapy. PSA progression was defined as a minimum of two increases in PSA, with the date of first increase (nadir + >0.02 ng/ml) as the progression date. For patients who received intermittent ADT, PSA increases during off-ADT periods were not counted as progression. TTP and TTM were censored on the date of last disease assessment for those who did not develop progression or metastasis. Demographic and treatment characteristics were compared across genotypes to assess for confounders using Kruskal-Wallis and Fisher's exact tests. Multivariable analyses were performed with Cox regression to adjust for known prognostic factors, including biopsy Gleason score, PSA at ADT initiation, age, and use of prior ADT with RT. P-values less than or equal to 0.05 were interpreted as statistically significant. Analyses were performed with SAS 9.4 (SAS Institute, Cary, NC, USA). Demographic and treatment factors are listed in the Table on page 39. The key outcomes are shown in the Figure on page 40. Although the crude time to progression and overall survival were worse with the *HSD3BI*(1245C) allele (particularly with two copies), there were no statistically significant differences in these endpoints on multivariable analysis. Notably, 105/213 (49%) of men had received prior ADT and 119/213 (56%) received an androgen receptor antagonist during salvage treatment, both of which may attenuate the effect of the variant allele. Nonetheless, we found a prominent difference in time to metastasis, with differences by genotype measured in years. Further details are included in the text of the second manuscript (Appendix 3).

What opportunities for training and professional development has the project provided?

One of the major goals of this project is to foster my training and educational development in prostate cancer research, and in this regard I have been very fortunate. Now at a critical point in my development as a young investigator, Dr. Nima Sharifi's ongoing mentorship has been invaluable. I have learned much through his modeling of the scientific, leadership, professional, and practical elements necessary for an investigator to contribute to the field. I am also thankful for the mentorship of Dr. Felix Feng, who has likewise been a role model and source of valuable advice.

Apart from mentorship, I have benefited from ongoing educational development and training through my involvement in multidisciplinary genitourinary conferences, prostate cancer research seminars, visiting professor sessions, radiation oncology grand rounds, and meetings with Dr. Sharifi and collaborators. Additionally, I have had the privilege of presenting our work at the 2016 and 2017 ASCO annual meetings, the 2016 Department of Defense IMPaCT meeting, and the 2016 and 2017 Prostate Cancer Foundation scientific retreats. These meetings have furnished excellent opportunities to network with other prostate cancer investigators and stay abreast of developments in the field.

Finally, I have had the privilege of caring for my own patients with prostate cancer, which has yielded experience and perspective that cannot be gained any other way, and which continually reaffirms my pledge to improve care for men with prostate cancer.

How were the results disseminated to communities of interest?

Please see above regarding presentation at national meetings. Please see Appendices 1-3 for published results.

What do you plan to do during the next reporting period to accomplish the goals?

We plan to expand our analyses by incorporating prospective data.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Please see “Research in context” box on the second page of the journal article in Appendix 1 and the “Key Points” box on the second page of the journal article in Appendix 3. In brief, we assessed whether there is a clinical impact of inheriting a variant form (allele) of the *HSD3B1* gene, previously shown to enable prostate cancer cells to make their own derivative of testosterone that can drive tumor progression, despite castration. We have demonstrated in three independent groups of men treated with androgen deprivation therapy (ADT) that the number of inherited copies of the variant allele corresponds to clinical outcomes (i.e. likelihood of disease progression, risk of metastasis (spread to the bones or other distant sites), and death). Men possessing the variant *HSD3B1* allele have worse clinical outcomes than men who have not inherited that allele. Moreover, men who have two copies of the variant allele tend to have profound resistance to ADT. In a fourth cohort of men who received ADT for biochemical failure after initial radiation therapy, we again found that the variant allele predicts for large differences in time to metastasis. Taken together, these results indicate that *HSD3B1* genotype is a powerful genetic biomarker of resistance to ADT, and can be used to identify men who might benefit from escalated therapy. Identification of these men has potential ramifications for guiding clinicians in augmenting standard ADT with highly-potent inhibitors of the androgen axis or chemotherapy in an effort to improve outcomes. Design and analysis of future studies should take into account *HSD3B1* genotype, in view of the differences in outcomes according to the number of variant alleles a man inherits.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

CHANGES/PROBLEMS

Nothing to report.

PRODUCTS

Publications, conference papers, and presentations

Journal publications

Hearn JWD, AbuAli G, Reichard CA, Reddy CA, Magi-Galluzzi C, Chang K-H, Carlson R, Rangel L, Reagan R, Davis BJ, Karnes RJ, Kohli M, Tindall D, Klein EA, and Sharifi N: HSD3B1 and Resistance to Androgen-Deprivation Therapy in Prostate Cancer: A Retrospective, Multicohort Study. *Lancet Oncology*; Oct;17(10): 2016; 1435-1444.

Federal funding support acknowledged.

Hearn JWD, Xie W, Nakabayashi M, Almassi N, Reichard CA, Pomerantz M, Kantoff PW, Sharifi N: *HSD3B1* Genotype and Response to Androgen Deprivation Therapy for Biochemical Recurrence after Radiotherapy for Localized Prostate Cancer. *JAMA Oncology*; doi:10.1001/jamaoncol.2017.3164. Published online October 12, 2017.

Federal funding support acknowledged.

Books or other non-periodical, one time publications

Nothing to report.

Other publications, conference papers, and presentations

Hearn JWD, AbuAli G, Reichard CA, Reddy CA, Magi-Galluzzi C, Chang K-H, Carlson R, Rangel L, Reagan R, Davis BJ, Karnes RJ, Kohli M, Tindall D, Klein EA, and Sharifi N: *HSD3B1* and Resistance to Androgen Deprivation Therapy in Prostate Cancer; Presented at the American Society of Clinical Oncology (ASCO) Annual Meeting, Chicago, IL, 2016. *J Clin Oncol* 34, 2016 (suppl; abstr 5015).*

Federal funding support acknowledged; *produced a manuscript

Hearn JWD, AbuAli G, Reichard CA, Reddy CA, Magi-Galluzzi C, Chang K-H, Carlson R, Rangel L, Reagan R, Davis BJ, Karnes RJ, Kohli M, Tindall D, Klein EA, and Sharifi N: HSD3B1 and Resistance to Androgen Deprivation Therapy in Prostate Cancer; Presented at the 2016 Department of Defense Innovative Minds in Prostate Cancer Today (IMPACT) meeting in Towson, Maryland.*

Federal funding support acknowledged; *produced a manuscript

Hearn JWD, AbuAli G, Reichard CA, Reddy CA, Magi-Galluzzi C, Chang K-H, Carlson R, Rangel L, Reagan R, Davis BJ, Karnes RJ, Kohli M, Tindall D, Klein EA, and Sharifi N: HSD3B1 and Resistance to Androgen Deprivation Therapy in Prostate Cancer; Presented at the 2016 Prostate Cancer Foundation Scientific Retreat in Carlsbad, California.*

Federal funding support acknowledged; *produced a manuscript

Hearn JWD, Xie W, Nakabayashi M, Almassi N, Reichard C, Pomerantz M, Kantoff P, Sharifi N: *HSD3B1* Genotype and Response to Androgen Deprivation Therapy for Biochemical Recurrence after Radiotherapy for Localized Prostate Cancer; Presented at the American Society of Clinical Oncology (ASCO) Annual Meeting, Chicago, IL, 2017. *J Clin Oncol* 35, no.15_suppl (May 2017) 5050-5050.*

Federal funding support acknowledged; *produced a manuscript

Hearn JWD, Xie W, Nakabayashi M, Almassi N, Reichard C, Pomerantz M, Kantoff P, Sharifi N: *HSD3B1* Genotype and Response to Androgen Deprivation Therapy for Biochemical Recurrence after Radiotherapy for Localized Prostate Cancer; Presented at the 2017 Prostate Cancer Foundation Scientific Retreat in Washington, D.C.*

Federal funding support acknowledged; *produced a manuscript

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name: Jason Hearn

Project Role: PI

Researcher Identifier (e.g. ORCID ID): Not applicable

Nearest person month worked: 6

Contribution to Project: Leading the project, coordinating components, data collection, analysis and interpretation, manuscript writing

Funding Support: This award

SPECIAL REPORTING REQUIREMENTS

Nothing to report.

APPENDICES

Appendix 1: Journal article detailing validation work (referenced above)

Appendix 2: Supplementary materials for the article in Appendix 1

Appendix 3: Journal article detailing analysis in the context of radiotherapy (referenced above)

HSD3B1 and resistance to androgen-deprivation therapy in prostate cancer: a retrospective, multicohort study



Jason W D Hearn*, Ghada AbuAli*, Chad A Reichard, Chandana A Reddy, Cristina Magi-Galluzzi, Kai-Hsiung Chang, Rachel Carlson, Laureano Rangel, Kevin Reagan, Brian J Davis, R Jeffrey Karnes, Manish Kohli, Donald Tindall, Eric A Klein, Nima Sharifi

Summary

Background *HSD3B1* (1245A>C) has been mechanistically linked to castration-resistant prostate cancer because it encodes an altered enzyme that augments dihydrotestosterone synthesis from non-gonadal precursors. We postulated that men inheriting the *HSD3B1* (1245C) allele would exhibit resistance to androgen-deprivation therapy (ADT).

Methods In this multicohort study, we determined *HSD3B1* genotype retrospectively in men treated with ADT for post-prostatectomy biochemical failure and correlated genotype with long-term clinical outcomes. We used data and samples from prospectively maintained prostate cancer registries at the Cleveland Clinic (Cleveland, OH, USA; primary study cohort) and the Mayo Clinic (Rochester, MN, USA; post-prostatectomy and metastatic validation cohorts). In the post-prostatectomy cohorts, patients of any age were eligible if they underwent prostatectomy between Jan 1, 1996, and Dec 31, 2009 (at the Cleveland Clinic; primary cohort), or between Jan 1, 1987, and Dec 31, 2011 (at the Mayo Clinic; post-prostatectomy cohort) and were treated with ADT for biochemical failure or for non-metastatic clinical failure. In the metastatic validation cohort, patients of any age were eligible if they were enrolled at Mayo Clinic between Sept 1, 2009, and July 31, 2013, with metastatic castration-resistant prostate cancer. The primary endpoint was progression-free survival according to *HSD3B1* genotype. We did prespecified multivariable analyses to assess the independent predictive value of *HSD3B1* genotype on outcomes.

Findings We included and genotyped 443 patients: 118 in the primary cohort (who underwent prostatectomy), 137 in the post-prostatectomy validation cohort, and 188 in the metastatic validation cohort. In the primary study cohort, median progression-free survival diminished as a function of the number of variant alleles inherited: 6·6 years (95% CI 3·8–not reached) in men with homozygous wild-type genotype, 4·1 years (3·0–5·5) in men with heterozygous variant genotype, and 2·5 years (0·7 to not reached) in men with homozygous variant genotype ($p=0\cdot011$). Relative to the homozygous wild-type genotype, inheritance of two copies of the variant allele was predictive of decreased progression-free survival (hazard ratio [HR] 2·4 [95% CI 1·1–5·3], $p=0\cdot029$), as was inheritance of one copy of the variant allele (HR 1·7 [1·0–2·9], $p=0\cdot041$). Findings were similar for distant metastasis-free survival and overall survival. The effect of the *HSD3B1* genotype was independently confirmed in the validation cohorts.

Interpretation Inheritance of the *HSD3B1* (1245C) allele that enhances dihydrotestosterone synthesis is associated with prostate cancer resistance to ADT. *HSD3B1* could therefore potentially be a powerful genetic biomarker capable of distinguishing men who are a priori likely to fare favourably with ADT from those who harbour disease liable to behave more aggressively, and who therefore might warrant early escalated therapy.

Funding Prostate Cancer Foundation, National Institutes of Health, US Department of Defense, Howard Hughes Medical Institute, American Cancer Society, Conquer Cancer Foundation of the American Society of Clinical Oncology, Cleveland Clinic Research Programs Committee and Department of Radiation Oncology, Gail and Joseph Gassner Development Funds.

Introduction

Nearly all prostate cancers express the androgen receptor, the importance of which is underscored by androgen-deprivation therapy (ADT), the most effective and widely used systemic therapy for prostate cancer for the past 70 years.¹ When combined with radiotherapy, ADT improves survival in selected patients.^{2,3} Similarly, ADT confers a survival advantage if given immediately after prostatectomy in node-positive disease.⁴ ADT is the cornerstone of treatment in men with metastatic disease,⁵ and has shown benefit even in the setting of biochemical failure after local therapy (ie, in the context of increasing prostate-specific antigen [PSA] after definitive surgery or

radiotherapy).⁶ For example, the TOAD trial⁷ published in 2016 showed improved survival with early versus delayed ADT in non-metastatic men with increasing PSA level, most of whom had biochemical failure after local therapy. Although nearly all men initially show a response to ADT, most will eventually develop castration-resistant prostate cancer. However, the duration of response to ADT varies substantially.^{8,9}

Progression from castration-sensitive to castration-resistant prostate cancer hinges on androgen receptor reactivation, which can occur by several mechanisms.^{10,11} Since the underlying processes typically emerge under selection pressure from ADT, knowledge of such processes

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Research in context

Evidence before this study

We searched PubMed for studies published between Jan 1, 1980, and Feb 1, 2016, on biomarkers of resistance to androgen-deprivation therapy (ADT) in prostate cancer. We used the search terms “androgen deprivation therapy” AND (“resistance” or “efficacy”), AND “prostate cancer”, AND “biomarker”. We identified 144 studies, common limitations of which included dependence on information not available at ADT initiation, emphasis on associations between polymorphisms with no known mechanistic correlate, absence of association with endpoints other than time to progression, and absence of validation. We restricted our search to studies that reported on people and were published in English.

Added value of this study

We tested the prespecified, mechanism-based hypothesis that men inheriting the *HSD3B1* (1245C) allele would exhibit innate resistance to ADT, since this allele had been established to increase intratumoural conversion of androgen precursor steroids to more potent androgens that can drive disease progression, despite castration. We demonstrated that *HSD3B1* genotype has a large effect on long-term clinical

endpoints, including progression-free survival, distant metastasis-free survival, and overall survival. Moreover, a stepwise difference was noted in outcomes according to the number of *HSD3B1* (1245C) alleles inherited, with differences measured in years. Additionally, we have validated the influence of *HSD3B1* genotype for progression-free survival by analysing three independent cohorts, representing both the post-prostatectomy and metastatic disease contexts. Finally, we found that *HSD3B1* genotype was associated with overall survival in both study cohorts (primary and metastatic validation cohorts) with mature follow-up.

Implications of all the available evidence

Our data show that *HSD3B1* genotype is a powerful genetic biomarker of resistance to ADT, and can be used to identify men who might benefit a priori from escalated therapy. This identification might have ramifications for selective early incorporation of highly-potent inhibitors of the androgen receptor axis, and could potentially inform selection of patients for chemohormonal therapy. Future studies should stratify by *HSD3B1* genotype in view of the differences in outcomes according to the number of variant alleles present.

generally cannot be used to establish a priori how patients will respond to this treatment. A major advance in the past decade has been the increased appreciation of intratumoural androgen synthesis.^{12,13} Before ADT, tumour androgen supply is dominated by gonadal testosterone. With gonadal suppression during ADT, the serum concentration of testosterone is greatly depleted, inhibiting tumour growth. However, proliferation can continue in the context of intratumoural androgen synthesis, mostly from adrenal precursor steroids and, possibly, at least in part, due to de-novo synthesis from cholesterol. Strong evidence for the importance of intratumoural androgen synthesis is found in the survival benefit from abiraterone (which depletes intratumoural androgens) and enzalutamide (which competes with intratumoural androgens).^{14–18} Additionally, transcripts for several steroidogenic enzymes—including *AKR1C3*, *HSD3B1*, and *HSD3B2*—are consistently upregulated in castration-resistant prostate cancer.¹⁹ In 2013, a mutation in *HSD3B1* was shown to provide a novel mechanism of resistance to ADT.²⁰ *HSD3B1* encodes 3 β -hydroxysteroid dehydrogenase-1 (3 β HSD1), an isoenzyme that is mainly expressed in peripheral tissues (eg, the prostate, skin, breast, and placenta), is responsible for catalysing the rate-limiting step in the conversion of adrenal androgen precursors to dihydrotestosterone, and is required for all pathways of dihydrotestosterone synthesis.²¹ *HSD3B1* (1245A>C) changes aminoacid 367Asn→Thr and renders 3 β HSD1 resistant to proteasomal degradation, causing substantial accumulation of this enzyme and, effectively, gain-of-function. The resultant increased intratumoural metabolic flux of adrenal precursors to more potent

androgens, such as dihydrotestosterone (the most potent androgen), therefore enhances androgen receptor activation and accelerates tumour proliferation, despite castration. Although the *HSD3B1* (1245C) allele can be acquired by mutation, it is also heritable in the form of a single nucleotide polymorphism (SNP; rs1047303) with allelic frequency of 15–35% in most cohorts (this frequency varies by ethnic origin, and is much lower in Asians and higher in white people).²²

At present, the clinical relevance of *HSD3B1* (1245C) inheritance in prostate cancer is unknown. One study²³ in China has linked this variant allele with increased progression to castration-resistant prostate cancer, but not with other endpoints. However, that study²³ was limited by the extremely low prevalence of the *HSD3B1* (1245C) allele in Chinese men, and therefore included no men homozygous for the variant allele and very few who had even one allele. Additionally, the study was confounded by the fact that nearly twice as many men included in the homozygous wild-type cohort started ADT when they already had metastatic disease.²³ As such, the potential clinical relevance of the variant allele remained undetermined.

Our present study was designed to specifically test the mechanism-based hypothesis that men inheriting the variant *HSD3B1* (1245C) allele would demonstrate evidence of intrinsic resistance to ADT, which would manifest as shorter time to progression, worse distant metastasis-free survival, and potentially worse overall survival than men not inheriting this variant. Moreover, on the basis of the number of variant alleles inherited, we hypothesised that men homozygous for the variant

allele would have the worst outcomes, men homozygous for the wild-type allele would have the best outcomes, and heterozygotes would potentially have intermediate outcomes.

Methods

Study design and participants

In this multicohort study, we used data from three large, prospectively maintained prostate cancer registries: the primary study cohort at Cleveland Clinic (Cleveland, OH, USA) and two validation cohorts from the Mayo Clinic (Rochester, MN, USA). For eligibility in the primary study cohort, men (of any age) had to have undergone prostatectomy at the Cleveland Clinic between Jan 1, and Dec 31, 2009, and to have been treated with ADT for biochemical failure. Biochemical failure was defined as a PSA value of 0.2 µg/L or higher, followed by a second higher reading (no specified time interval between the two readings), or one PSA value of 0.5 µg/L or higher.²⁴ We specified this definition to reduce the likelihood of inadvertently including men with low detectable concentration of PSA produced from residual benign tissue or biologically indolent cancer, since the inclusion of such patients would undermine the study's ability to test the mechanism-based hypothesis. Patients with minimally detectable PSA coming from benign tissue rather than from cancer would not have progression, metastasis, or death from cancer (because they had no cancer). Patients with a minute amount of very indolent cancer such that they never met the definition of biochemical failure would be less likely to progress, metastasise, or die from cancer than those whose cancer proliferated fast enough that they met the definition of biochemical failure.

Additionally, men who received postoperative adjuvant or salvage radiotherapy were eligible for the study, if they had residual active disease as indicated by continued increase in their PSA after treatment (ie, PSA after radiotherapy higher than postoperative preradiotherapy value). Patients were excluded if the quality of their sample of DNA was insufficient to permit genotyping.

For the post-prostatectomy validation cohort, we used patient data from the National Cancer Institute-funded Sponsored Programs of Research Excellence's (SPORE) database at the Mayo Clinic, and included men who underwent prostatectomy between Jan 1, 1987, and Dec 31, 2011. We identified and included all men who had received ADT for biochemical or non-metastatic clinical failure (eg, local failure) after prostatectomy and had blood samples available for research. For the metastatic validation cohort, we queried a large Mayo Clinic registry of patients with advanced prostate cancer. We identified men enrolled between Sept 1, 2009, and July 31, 2013, with metastatic castration-resistant prostate cancer who had blood samples banked for research.

To minimise confounding, we excluded patients from the metastatic validation cohort if they had received radiotherapy for initial local therapy (since use of ADT

with radiotherapy was not adequately captured in this cohort) or if they had confirmed or probable neuroendocrine prostate cancer (given that such cancers are generally not dependent on the androgen receptor axis). We used no age restrictions for patients in the validation cohorts. All patients were aged older than 18 years.

All biological samples and clinical data were obtained with written informed patient consent as part of informed consent protocols approved by local institutional review boards at the Cleveland Clinic and the Mayo Clinic.

Procedures

We reviewed prostatectomy specimens from the primary cohort to obtain cores of non-neoplastic tissue for germline genotyping of all patients in the primary cohort. A genitourinary pathologist (CM-G) reviewed each of these specimens to identify non-neoplastic tissue. Genotyping was accomplished with a melting assay (appendix pp 1, 2, 7). We used peripheral blood mononuclear cells to genotype the two validation cohorts using the melting assay. *HSD3B1* has one homologue (*HSD3B2*) and four non-processed pseudogenes (*HSD3BP1*, *HSD3BP2*, *HSD3BP4*, and *HSD3BP5*), which have very closely related DNA sequences that might obscure detection of the variant sequence.²¹ We therefore developed a melting assay using an unlabelled, locked, nucleic acid oligonucleotide probe in an asymmetric PCR to complete targeted genotyping at this locus for all cohorts (appendix pp 1, 7). All patients were identified in all cohorts (by CAREd, JWDH, RC, LR, and MK) before determination of genotype, and tissue processing and genotyping were completed by investigators who were masked to clinical data. Data collection and analysis were uncoupled, because the study statistician (CAREd) was not involved in collection of clinical data or genotyping.

See Online for appendix

Outcomes

The primary endpoint was progression-free survival. Progression was an investigator-assessed composite endpoint defined as the first occurrence of a second increase in PSA on ADT (no absolute threshold), radiographic or clinical progression (defined as the development of a local or regional nodal recurrence on physical examination, CT, or MRI; distant metastasis seen on bone scan, CT, or MRI; or death from prostate cancer), or initiation of any second-line therapy. The secondary endpoints were distant metastasis-free survival (ie, the probability of surviving without distant metastases) and overall survival. Time-to-event outcomes were measured from the date when ADT was initiated.

Statistical analysis

We analysed progression-free survival, distant metastasis-free survival, and overall survival using Kaplan-Meier methods. We used the log-rank test for trend with one degree of freedom to assess potential gene-dose

effects across the three genotypes. We did Cox proportional hazards regression to further assess potential allele–dose effects. We compared demographic and treatment characteristics across genotypes to assess for confounders using Fisher's exact test and the Kruskal-Wallis one-way analysis of variance. We used Fisher's exact test rather than the χ^2 test because the latter is less accurate if the number of patients in a category is low (ie, lower than five). We used multivariable Cox proportional hazards models to account for potential

confounders. Covariates included common clinico-pathological variables known to correlate with clinical outcomes, including American Joint Commission on Cancer T stage, N stage, PSA at initiation of ADT, and Gleason score. We examined T stage as a categorical variable using all T stages in two separate models. In the first model, stage T2 was the reference group, examining stage 3a versus stage 2 and 3b–4 versus 2. In the second model, stage T3a was the reference group, examining stage 3b–4 versus 3a and 2 versus 3a. Stage T4 was combined with stage T3b because there was only one stage T4 patient in our study population. Stage T3b–4 is typically more predictive of poor clinical outcomes than is T3a, and therefore we did not combine all T3 patients into one category. All tests were two-sided, and p values less than or equal to 0.05 were judged to be statistically significant. We used SAS version 9.3 for our statistical analyses.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. JWDH, GA, CAREi, CAREd, CM-G, RC, LR, MK, DT, and NS had access to all the raw data. After extensive quality assurance and data verification, all authors vouched for the completeness and integrity of the data and statistical analysis. The corresponding author had full access to all data and had the final responsibility for the decision to submit for publication.

Results

In the primary Cleveland Clinic cohort, we identified 177 eligible men who met study criteria and underwent prostatectomy between Jan 1, 1996, and Dec 31, 2009. Of these, 118 (67%) were successfully genotyped and could be analysed, which is indicative of the well-established challenge of genotyping with formalin-fixed paraffin-embedded tissue.^{25–27} Our melting analysis technique was able to reliably distinguish genotypes (appendix p 2), and was validated with Sanger sequencing for 60 samples (20 for each genotype) with 100% concordance.

Of the genotyped men in the primary cohort, *HSD3B1* (1245C) allelic frequency was 36% (86 of 236 alleles); 44 (37%) of 118 men were homozygous wild-type, 62 (53%) were heterozygous, and 12 (10%) were homozygous variant. No significant differences were noted in demographic or treatment characteristics of participants by genotype, although the crude proportions of T3b–4 tumours and Gleason score 8–10 disease were highest in homozygous variant men (table). Multivariable analyses were used to adjust accordingly for these differences.

Of 140 patients in the post-prostatectomy validation cohort (the Mayo Clinic SPOR registry) who met study criteria, 137 (98%) were successfully genotyped and could be analysed. *HSD3B1* (1245C) allelic frequency was 26% (70 of 274 alleles), and the genotype distribution was as follows: 77 (56%) of 137 men were homozygous wild-type,

	Homozygous wild-type patients (n=44)	Heterozygous patients (n=62)	Homozygous variant patients (n=12)	p value*
Follow-up, years	7.1 (4.0–9.0)	6.7 (4.1–9.0)	6.2 (3.6–6.8)	0.36
Age, years	65 (60–70)	65 (55–70)	63 (57–67)	0.36
Ethnic origin				0.16
White	34 (77%)	56 (90%)	11 (92%)	
Black	8 (18%)	4 (6%)	0	
Hispanic	0	1 (2%)	0	
Asian	1 (2%)	0	0	
Other	1 (2%)	1 (2%)	1 (8%)	
Pathological T stage†				0.056
T2	14 (32%)	9 (15%)	0	
T3a	13 (30%)	17 (27%)	4 (33%)	
T3b–4‡	17 (39%)	36 (58%)	8 (67%)	
Pathological N stage†				0.97
N0	30 (68%)	39 (63%)	9 (75%)	
N1	10 (23%)	16 (26%)	2 (17%)	
NX	4 (9%)	7 (11%)	1 (8%)	
Prostate-specific antigen at ADT initiation (µg/L)	3.3 (0.8–8.0)	1.5 (0.6–5.4)	1.9 (1.1–7.3)	0.33
Gleason score				0.42
6	2 (5%)	0	0	
7	22 (50%)	35 (57%)	5 (42%)	
8–10	20 (46%)	27 (44%)	7 (58%)	
ADT type				0.69
GnRH agonist or orchiectomy	41 (93%)	56 (90%)	12 (100%)	
Androgen receptor antagonist	3 (7%)	6 (10%)	0	
ADT use				0.23
Continuous	25 (57%)	27 (44%)	8 (67%)	
Intermittent	19 (43%)	35 (57%)	4 (33%)	
Adjuvant or salvage radiation therapy				0.66
No	31 (71%)	38 (61%)	8 (67%)	
Yes	13 (30%)	24 (39%)	4 (33%)	
Neoadjuvant treatment				0.97
No	31 (70%)	45 (73%)	9 (75%)	
ADT	10 (23%)	14 (23%)	2 (17%)	
Chemotherapy or immunotherapy	3 (7%)	3 (5%)	1 (8%)	

Data are median (IQR) or n (%). ADT=androgen-deprivation therapy. GnRH=gonadotropin-releasing hormone.

*p values are comparisons across the three genotypes. †Pathological stage refers to the American Joint Commission on Cancer's tumour–node–metastasis staging system.²⁸ ‡Stages T3b and T4 were combined because only one patient (a heterozygote) was stage T4 in our study population.

Table: Baseline characteristics of the primary cohort by *HSD3B1* genotype

50 (37%) were heterozygous, and ten (7%) were homozygous variant (for demographic and treatment characteristics see appendix p 8). Of 204 patients who met study criteria and were in the metastatic validation cohort (Mayo Clinic metastatic prostate cancer registry) between Sept 1, 2009, and July 31, 2013, 188 (92%) patients were successfully genotyped and could be analysed. These men had initially been diagnosed with prostate cancer between Jan 1, 1983, and July 31, 2012. *HSD3B1* (1245C) allelic frequency was 27% (101 of 376 alleles), and the genotype distribution was as follows: 98 (52%) of 188 men were homozygous wild-type, 79 (42%) were heterozygous, and 11 (6%) were homozygous variant (for demographic and treatment characteristics see appendix p 9). Pooling data from all three cohorts, the overall *HSD3B1* (1245C) allelic frequency was 29% (257 of 886 alleles), and the pooled genotype distribution was as follows: 219 (49%) of 443 men were homozygous wild-type, 191 (43%) were heterozygous, and 33 (7%) were homozygous variant.

In both validation cohorts, the demographic and treatment characteristics of the patients did not differ according to genotype (appendix pp 8–9), with the exception of N-stage in the Mayo Clinic post-prostatectomy SPORC cohort. The distribution of N1 disease in this cohort was five (50%) of ten men in the homozygous variant group, compared with ten (13%) of 77 men in the homozygous wild-type and eight (16%) of 50 men in the heterozygous group ($p=0.025$). This discrepancy was adjusted for during multivariable analysis. Median follow-up in this cohort (4.9 years [IQR 2.6–7.6]) was shorter than for the primary cohort (6.7 years [3.9–8.9]; table) and the Mayo metastatic validation cohort (6.0 years [3.1–9.2]), and was especially short in the homozygous variant group (3.2 years [1.8–4.6]). Differences in the duration of follow-up between the three genotypes were taken into account with the use of Cox proportional hazards regression. In view of the low number of metastatic and death events during the short observation period (appendix p 3), the post-prostatectomy validation cohort was used to validate the effect of *HSD3B1* genotype on only progression-free survival. By contrast, the median follow-up from ADT initiation for the metastatic validation cohort was sufficient to enable analysis of both progression-free survival and overall survival. Distant metastasis-free survival was not calculated in this cohort, since these were patients who already had metastasis. Chemotherapy was commonly used for castration-resistant prostate cancer, and its use was similar across the three genotypes in the metastatic cohort (57% [56 of 98] in homozygous wild-type men, 61% [48 of 79] in heterozygotes, and 55% [six of 11] in homozygous-variant men [$p=0.83$]; appendix p 9). Docetaxel was used in 106 (96%) of the 110 patients who received chemotherapy in this cohort.

Progression-free survival was significantly associated with *HSD3B1* genotype in the primary study cohort

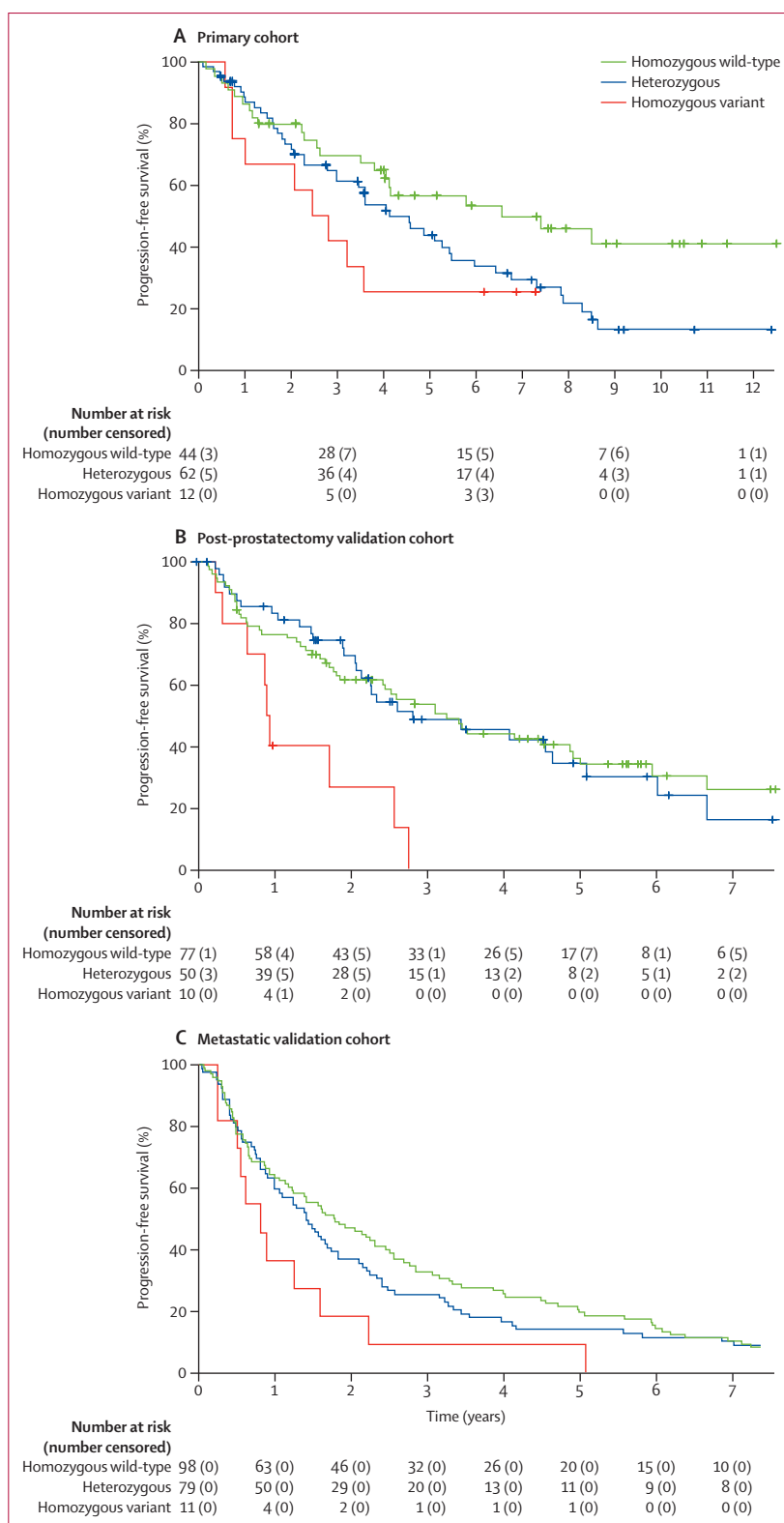


Figure 1: Progression-free survival according to *HSD3B1* genotype

Progression-free survival according to *HSD3B1* genotype in (A) the primary study cohort, (B) the post-prostatectomy validation cohort, and (C) the metastatic validation cohort.

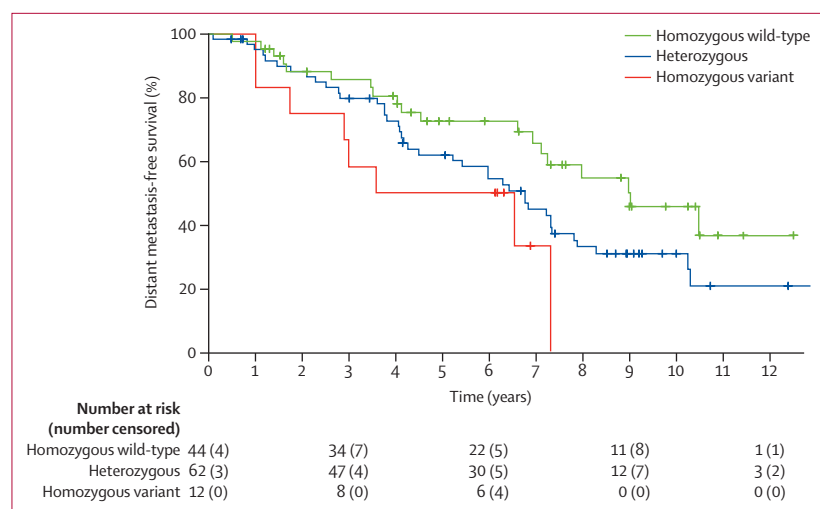


Figure 2: Distant metastasis-free survival in the primary study cohort

(figure 1A), and diminished as a function of the number of *HSD3B1* (1245C) alleles inherited: median 6.6 years (95% CI 3.8–not reached) in homozygous wild-type men; 4.1 years (3.0–5.5) in heterozygous men; and 2.5 years (0.7–not reached) in homozygous variant men ($p=0.011$). Relative to the homozygous wild-type genotype, inheritance of two copies of the variant allele was predictive of decreased progression-free survival (hazard ratio [HR] 2.4 [95% CI 1.1–5.3], $p=0.029$). Additionally, inheritance of one copy of the variant allele was also associated with decreased progression-free survival (HR 1.7 [95% CI 1.0–2.9], $p=0.041$). On multivariable analysis (appendix p 10), the HR for progression was 1.6 (95% CI 1.0–2.7, $p=0.074$) for men with at least one variant allele, which compared favourably with Gleason score (for Gleason score 8–10 vs 6–7: HR 1.3 [95% CI 0.8–2.0], $p=0.31$). The distribution of each type of progression event (first occurrence of a second increase in PSA on ADT, radiographic or clinical progression, or initiation of second-line therapy) is described in the appendix (p 11).

In the post-prostatectomy validation cohort, progression-free survival was associated with *HSD3B1* genotype (figure 1B). Median progression-free survival was 3.3 years (95% CI 1.9–4.9) in homozygous wild-type men, 2.8 years (2.1–5.1) in heterozygous men, and 0.9 years (0.2–2.6) in homozygous variant men ($p=0.0022$). Homozygous variant men had an increased risk of progression compared with homozygous wild-type men (HR 3.4 [95% CI 1.6–7.0], $p=0.0013$), whereas progression-free survival did not differ significantly between heterozygous men and the wild-type group (HR 1.0 [0.7–1.7], $p=0.85$). The effect of homozygous variant genotype on progression-free survival persisted with prespecified adjustment for lymph node status (lymph node-negative vs lymph node-positive; HR 2.7 [95% CI 1.2–5.9], $p=0.013$), whereas heterozygotes did not differ significantly from homozygous wild-type men (1.0 [0.6–1.6], $p=0.98$).

Median progression-free survival in the metastatic validation cohort was also associated with *HSD3B1* genotype (figure 1C). As in the other two cohorts, the largest difference was between homozygous wild-type (median 1.8 years [95% CI 1.2–2.5]) and homozygous variant men (0.8 years [0.3–1.6], $p=0.024$). The corresponding HR for progression was 2.0 (95% CI 1.1–3.8, $p=0.027$). Men with heterozygous genotype had an intermediate progression-free survival (1.4 years [95% CI 1.0–1.8], HR for progression 1.1 [0.8–1.5], $p=0.38$).

Distant metastasis-free survival was assessable only in the primary study cohort because of inadequate length of follow-up in the post-prostatectomy validation cohort and the presence of metastatic disease at ADT initiation in the metastatic cohort. Distant metastasis-free survival was therefore not validated in any external cohorts. Distant metastasis-free survival was significantly associated with *HSD3B1* genotype in the primary study cohort and decreased according to the number of variant alleles inherited: median 9.1 years (95% CI 7.4–not reached) in homozygous wild-type men; 6.8 years (4.3–7.4) in heterozygotes; and 3.6 years (1.0–7.3) in homozygous variant men ($p=0.014$; figure 2). Compared with the homozygous wild-type genotype, inheritance of two copies of the variant allele was predictive of worse distant metastasis-free survival (HR 2.7, 95% CI 1.2–6.2, $p=0.022$). The corresponding HR for men with heterozygous genotype (ie, one copy of the variant allele) was 1.7 (95% CI 1.0–2.8, $p=0.074$). The effect of both homozygous variant genotype and heterozygous genotype on distant metastasis-free survival was confirmed on multivariable analysis (appendix p 10).

Overall survival was significantly associated with *HSD3B1* genotype in both of the cohorts with sufficiently mature follow up to enable its analysis (the primary and metastatic validation cohorts). Figure 3A shows a stepwise reduction in overall survival as a function of the number of variant alleles inherited in the primary cohort. The 5-year point estimates of overall survival were 82% (95% CI 69–94) in homozygous wild-type men, 74% (62–85) in heterozygous men, and 58% (30–86) in homozygous variant men; and 10-year point estimates of overall survival were 55% (95% CI 35–75) in homozygous wild-type men, 35% (21–49) in heterozygous men, and 0% (0–0) in homozygous variant men ($p=0.0064$). Median overall survival in the primary cohort was 16.7 years (95% CI 7.9–16.7) in homozygous wild-type men, 7.4 years (6.4–9.3) in heterozygous men, and 7.3 years (3.8–8.1) in homozygous variant men ($p=0.0064$). Relative to the homozygous wild-type genotype, inheritance of two copies of the variant allele was predictive of worse overall survival (HR 3.3 [95% CI 1.3–8.3], $p=0.013$). For men with the heterozygous genotype, the inheritance of one copy of the variant allele was also predictive of worse overall survival (HR 2.0 [1.1–3.7], $p=0.036$). Multivariable analysis confirmed the

effect of *HSD3B1* genotype on overall survival (appendix p 10).

Similarly, in the metastatic validation cohort, median overall survival from the time of ADT initiation was reduced according to the number of variant alleles present. The 5-year point estimates of overall survival were 72% (95% CI 63–82) in homozygous wild-type men, 62% (51–73) in heterozygous variant men, and 46% (16–75) in homozygous variant men; and 10-year point estimates of overall survival were 49% (95% CI 38–60) in homozygous wild-type men, 25% (14–36) in heterozygous men, and 16% (0–39) in homozygous variant men ($p=0.0042$). Median overall survival in the metastatic validation cohort was 9.7 years (95% CI 6.7–12.1) in homozygous wild-type men, 6.8 years (5.2–8.0) in heterozygous variant men, and 4.6 years (1.6–7.5) in homozygous variant men ($p=0.0042$; figure 3B). Compared with men with homozygous wild-type genotype, the HR for death was 2.5 (95% CI 1.2–5.0, $p=0.013$) in homozygous variant men and 1.5 (1.0–2.1, $p=0.036$) in heterozygotes. Outcomes for each cohort with all genotypes combined are in the appendix (pp 4–6). Prostate cancer-specific survival for the primary cohort (which had data for cause of death) is shown in the appendix (p 6). As with overall survival, prostate cancer-specific survival diminished according to *HSD3B1* inheritance between the three genotypes ($p=0.029$). Compared with the homozygous wild-type reference group, the HR for prostate cancer-specific mortality was 3.1 (95% CI 1.1–8.7; $p=0.027$) in homozygous variant men, and was 1.7 (0.8–3.3; $p=0.17$) in heterozygotes. Overall survival could not be meaningfully analysed in the SPOR post-prostatectomy cohort due to inadequate length of follow-up.

Discussion

Our results show that inheritance of the *HSD3B1* (1245C) allele (homozygous variant and heterozygotes) was associated with decreased progression-free survival, distant metastasis-free survival, and overall survival, with differences measured in years. These results are biologically credible, because tumour cells carrying the *HSD3B1* (1245C) allele are able to more efficiently produce their own dihydrotestosterone than those without this allele. The observed stepwise decrease in all three endpoints would be unlikely to occur by chance, and strongly suggests allele dose-dependence. Additionally, validation of our initial findings in two independent cohorts for progression-free survival supports the effect of *HSD3B1* (1245C) inheritance on resistance to ADT. Genotype might also be prognostic irrespective of ADT, although the mechanistic underpinnings suggest that the associated growth advantage would be most pronounced with ADT.

As an extension of our preclinical work that identified an association between *HSD3B1* (1245C) and increased 3 β HSD1 enzyme concentrations, we previously assessed

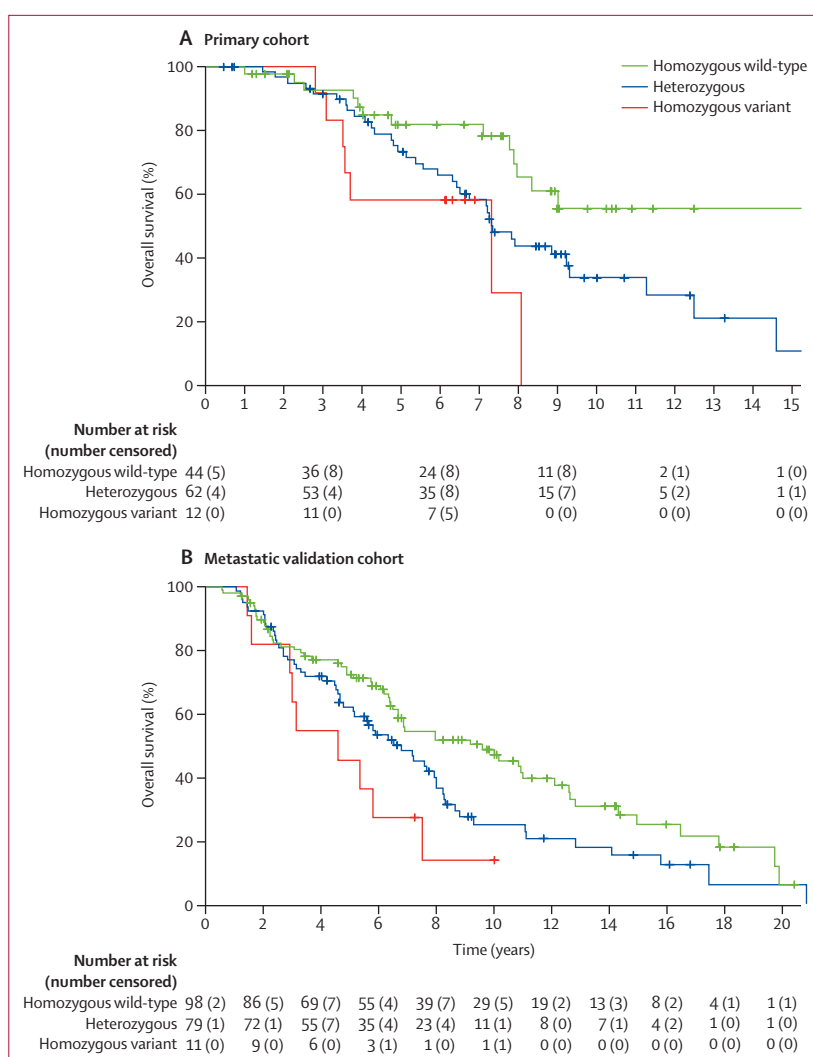


Figure 3: Overall survival

Overall survival according to *HSD3B1* genotype in (A) the primary study cohort and (B) the metastatic validation cohort.

tumours in men with castration-resistant prostate cancer.²⁰ In homozygous variant tumours, we noted a robust increase in 3 β HSD1 enzyme concentrations, whereas we did not record any such clear increase in heterozygous variant tumours.²⁰ However, expression level was measured in only two of these tumours in the heterozygous variant group, meaning that we could not make a definitive conclusion regarding the importance of heterozygous inheritance. By contrast, in this study, we analysed 191 heterozygotes, with most of our findings suggesting that heterozygous inheritance is biologically relevant. Progression-free survival in the post-prostatectomy validation cohort (figure 1B) is the only set of Kaplan-Meier curves among the six plots that does not suggest a difference between the heterozygous and homozygous wild-type genotypes, and this cohort had the shortest follow-up. Apart from the question of whether or not an intermediate increase in enzyme

concentration is associated with a single variant allele, our previous data²⁰ showed that loss of heterozygosity of the wild-type allele occurs under selective pressure from ADT. Three (27%) of 11 patients with germline heterozygous inheritance had developed castration-resistant prostate cancer tumours with loss of heterozygosity of the wild-type allele, whereas no patient had lost the variant allele.²⁰ Loss of heterozygosity of the wild-type allele in tumours could probably contribute to the difference in outcomes between men with the heterozygous genotype and those with the homozygous wild-type genotype. The discordance between germline inheritance and tumour DNA is indicative of somatic alterations, which are known to increase as tumours evolve, especially under selective pressure from ADT. Our previous work²⁰ showed that even homozygous wild-type men can eventually acquire the variant allele (three [12%] of 25 men assessed). Tumour DNA from castration-resistant prostate cancer is difficult to obtain in large numbers of patients and was unavailable from the cohorts in this study. Nonetheless, we anticipate that future studies will take somatic alterations in *HSD3B1* into account with analysis of tumour DNA to demonstrate an even stronger association between genotype and clinical outcomes.

Since Huggins and Hodges published their seminal work on the therapeutic effects of castration in 1941,¹ ADT has been the cornerstone of systemic therapy for prostate cancer. So far, prediction of innate resistance to ADT has been challenging. A previous study²⁹ analysed 109 SNPs in several steroidogenic genes, including *HSD3B1*, and reported no clear association with biochemical recurrence in men with resected prostate cancer. However, most patients analysed in the primary cohort in that study²⁹ were not treated with ADT. This difference is crucial in relation to our present study, since the growth advantage of the variant allele would be most pronounced under conditions of castration, as previously noted. Furthermore, *HSD3B1* has one homologue (*HSD3B2*) and four non-processed pseudogenes, which have very closely related DNA sequences that can obscure detection of the variant sequence. The accuracy of high-throughput genotyping techniques, including the methods used in multi-SNP analyses, can be adversely affected by such similar sequences and is highly dependent on optimal primer design.^{30,31} Such concerns provided the motivation for our development and validation of the specific high-resolution melting analysis technique we used in this study, since accurate genotyping was crucial.

Another previous study³² associated a non-coding SNP 13 kb upstream of *HSD3B1* (rs1856888) with development of castration-resistant prostate cancer. This SNP might affect the expression of *HSD3B1* or have an independent regulatory role. Alternatively, the correlation between this SNP and castration-resistant prostate cancer might be due to its proximity to *HSD3B1* and the biochemical activity that *HSD3B1* confers. Several other studies^{33–35}

have shown an association between germline variants in transmembrane steroid transporters and the development of castration-resistant prostate cancer. Therefore, other germline variants may confer additional information in combination with *HSD3B1* (1245C).

The ramifications of a biomarker able to predict ADT resistance are far-reaching. Our findings suggest *HSD3B1* genotype could a priori distinguish men with disease likely to respond favourably to ADT from those with disease prone to behave more aggressively, and who, therefore, might merit escalated therapy. Combined androgen blockade with the upfront addition of an androgen receptor antagonist to castration has long been debated, but analyses of several trials suggest that the clinical benefit of combined androgen blockade in unselected patients is small at best.³⁶ Although speculative, a differential benefit might be possible from use of combined androgen blockade, with little incremental gain for men who are homozygous wild-type, but meaningful use in men possessing the variant allele. *HSD3B1* (1245C) genotype could also guide future studies in terms of selective early incorporation of highly potent inhibitors of the androgen receptor axis, such as enzalutamide or abiraterone acetate. Men with the *HSD3B1* (1245C) allele, especially two copies (homozygotes), could potentially benefit greatly if one of these drugs were started with ADT rather than waiting until development of castration-resistant prostate cancer. With respect to the possible use of such drugs for men who inherit the *HSD3B1* (1245C) allele, abiraterone is clinically converted by 3 β HSD to the more potent anti-androgen metabolite D4A,³⁷ which in turn is further converted to other metabolites in patients, including 3-keto-5 α -abiraterone which stimulates the androgen receptor.³⁸ Nonetheless, the ultimate clinical effect of these steroidal metabolites of abiraterone remains to be determined.

Similar reasoning would suggest *HSD3B1* genotype could be informative in decisions regarding chemohormonal therapy. Two landmark trials, the Eastern Cooperative Oncology Group E3805 (CHAARTED)³⁹ and STAMPEDE,⁴⁰ showed that use of chemohormonal therapy before development of castration-resistant prostate cancer substantially improved survival outcomes compared with ADT alone. Cytotoxic therapy would probably be most beneficial in men who are least likely to have a durable response to ADT, whereas men who are likely to have a sustained response to ADT might benefit to a lesser extent. *HSD3B1* genotype could therefore help to guide patient management, especially for men with a marginal ability to tolerate chemotherapy.

The *HSD3B1* genotype might be influential in several other domains, one of which is the combination of ADT with radiotherapy. Additionally, there are potential implications for more refined identification of men for active surveillance. Active surveillance might have an increased risk in men with homozygous variant *HSD3B1* (1245C) inheritance, since they are not likely to respond durably to ADT, which would be their recourse if the

window for curative local therapy were missed. By contrast, homozygous wild-type inheritance might reinforce a recommendation for active surveillance. Similarly, *HSD3B1* genotype could also be informative in consideration of salvage radiotherapy for patients. Our results also provide insight regarding the potential effect of pharmacological inhibition of 3β HSD1. Because of the quite low prevalence of the homozygous variant genotype, *HSD3B1* (1245C) as a biomarker could be used in the clinic as a binary factor—no variant alleles versus one or more variant alleles. We believe it was informative to analyse the three groups of genotypes separately, since the patients with homozygous variant genotype seem to fare especially poorly in terms of their rate of progression, metastasis, and death; however, we acknowledge that the clinical implications might be similar for one versus two variant alleles in some clinical situations.

Our study has several limitations. Each of the three cohorts had a modest sample size, although the similarity of results in the three cohorts and the evidence of allele dose dependence strongly support the effect of *HSD3B1* (1245C) inheritance. Nonetheless, future work to prospectively assess the role of *HSD3B1* will be a high priority, in view of the retrospective design of our study. Correlation of serum and tissue steroid profiles with genotype will also be informative. The prevalence of each genotype varied across our three cohorts (homozygous wild-type 37–56%, heterozygous variant 36–52%, and homozygous variant 6–10%). Apart from random variation, the allelic distribution might be somewhat different in the populations sampled, even in the subsets of white patients. Future studies are needed to provide additional information to refine prevalence estimates. Further analysis of patients of other ethnic origins would also be of value in view of the under-representation of these men in our cohorts, although the lower allelic frequency in non-white populations might be challenging.

In this study we evaluated two cohorts with biochemical failure and found *HSD3B1* genotype to be associated with outcomes in this setting. However, we were only able to meaningfully analyse distant metastasis-free survival in the primary cohort because of short follow-up duration in the post-prostatectomy validation cohort. We also explored whether men at the opposite end of the disease scale (ie, the validation cohort of men who ultimately developed metastatic castration-resistant prostate cancer) had different outcomes according to *HSD3B1* status, and our data showed that they did. However, the more general question of whether the same is true of an unselected population of men with metastatic prostate cancer warrants further investigation.

One other potential limitation of our study was that a uniform follow-up schedule was not used. Systematic differences in assessment intervals can lead to spurious results (eg, in comparison of progression-free survival across trials with different assessment schedules).^{41,42} However, in this study, assessment frequency was at the

discretion of the treating physicians for all patients without regard to genotype (genotype was unknown at the time of clinical decision making). Thus, although this variation in assessment frequency should be considered when comparing our cohorts with each other or with additional cohorts, it would not reduce the validity of comparing men by genotype within each of our study cohorts, which was our objective.

In conclusion, our findings in three independent cohorts nominate germline *HSD3B1* genotype as a genetic biomarker of resistance to ADT for prostate cancer, and are concordant with what would be predicted from the underlying molecular mechanism.

Contributors

JWDH, GA, K-HC, and NS conceived and designed the study and developed the methods. JWDH, GA, CAREi, CM-G, RC, LR, MK, DT, EAK, and NS acquired data. JWDH, CAREd, and NS analysed and interpreted the data. KR, BJD, RJK, MK, DT, EAK, and NS provided administrative, technical, or material support. JWDH, CAREd, MK, DT, EAK, and NS provided study supervision. All authors wrote and provided final approval of the manuscript.

Declaration of interests

A patent for 3β -hydroxysteroid dehydrogenase in steroid-dependent disease has been filed by Cleveland Clinic. All grant support and other funding is listed in the Acknowledgments section. GA, CAREi, CAREd, CM-G, K-HC, RC, LR, KR, BJD, RJK, and EAK declare no competing interests.

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed.
We post it as supplied by the authors.

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Appendix

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Supplementary Methods

DNA extraction, amplification, and genotyping.

The DNA for the primary cohort was extracted from 3 freshly cut 10µm sections of benign prostatectomy tissue using QIAamp DNA FFPE Tissues kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. The DNA quantity and quality was measured using NanoDrop (Thermo Scientific, Waltham, MA, USA). DNA from the Mayo cohorts was extracted from peripheral blood mononuclear cells. Genomic DNA was stored at no more than 4°C. The PCR was performed in 20µL reactions (final volume) using LightScanner Master Mix (Biofire, Salt Lake City, UT, USA), 4µL extracted genomic DNA (10µg/µL), forward primer, reverse primer, and unlabeled LNA probe at concentrations of 0.05, 0.5, and 0.5µM, respectively (Table S1). The PCR and the melting analyses were performed in StepOnePlus™ System (Life Technologies, Grand Island, NY, USA) using the following conditions: 2 min at 95°C, followed by 60 cycles^{1,2} of 94°C for 30 s, 66°C for 30 s, and 75°C for 30 s. The amplification cycles were then followed by the melting steps: 95°C for 30 s, followed by cooling to 25°C for 60 s, and a slow final denaturation to 95°C at a thermal ramp rate of 0.3%. Data were analyzed using probe melting derivative peaks (Figure S1). Each validation run was performed with duplicate control samples representing the three different genotypes and each sample was tested in duplicate during the study genotyping.

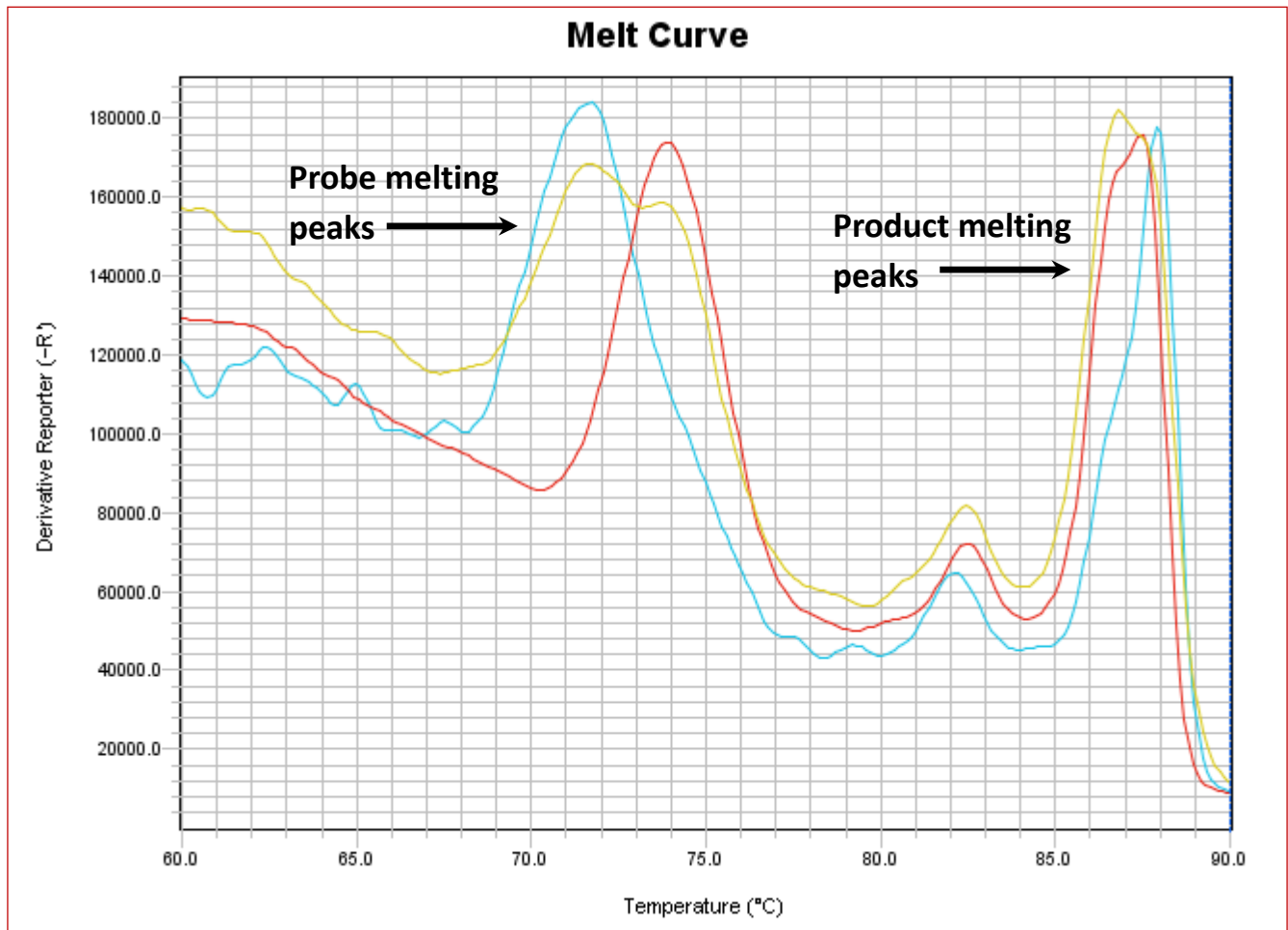


Figure S1: Derivative melting curves of homozygous wild-type (red), homozygous variant (blue), and heterozygous (yellow). In a homozygous variant sample, the probe shows a melting peak at a temperature that is 2°C lower than the probe's melting temperature in a wild-type sample. The heterozygous sample shows two melting peaks, whilst the full length product in all samples melts at a higher temperature (85°C-90°C).

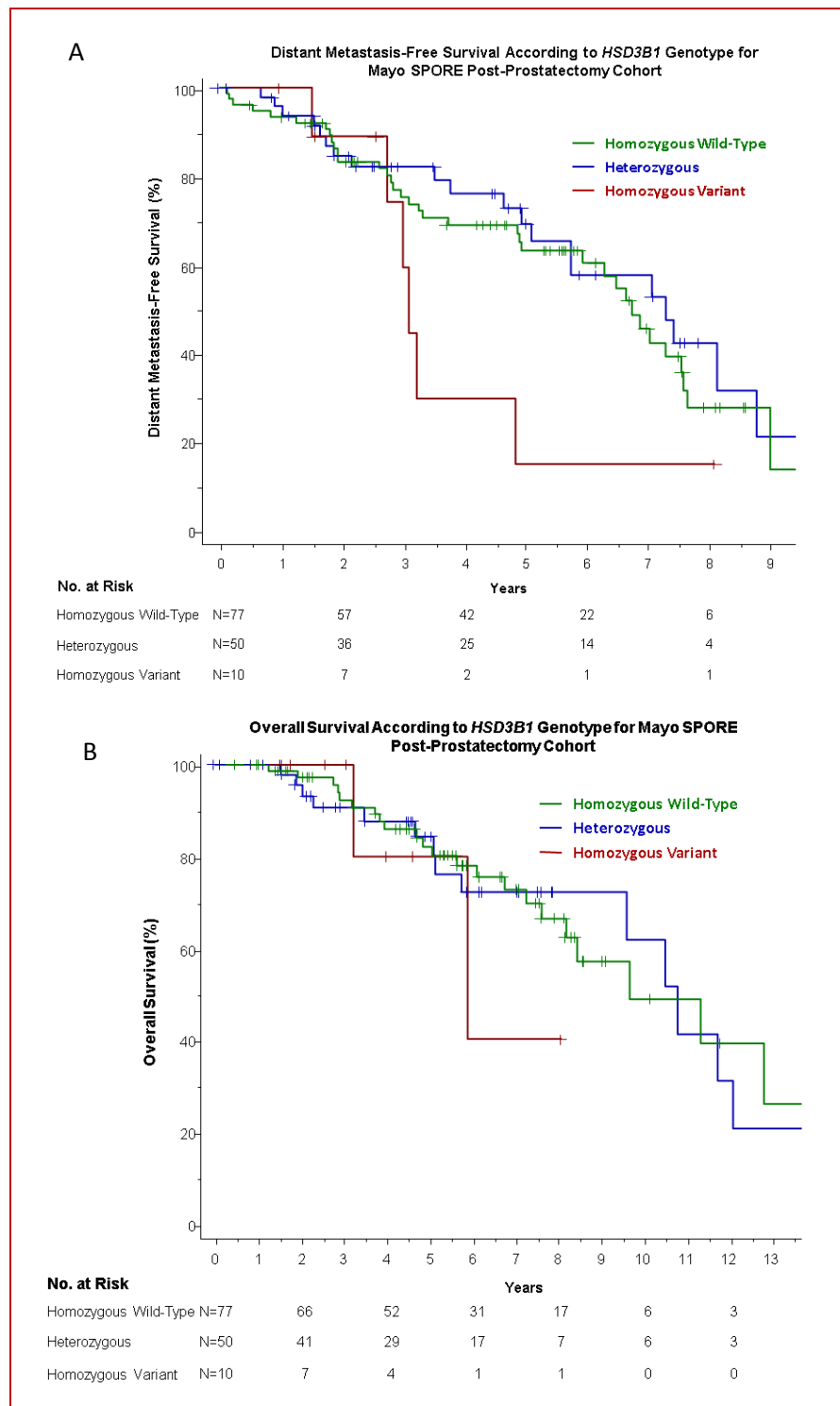


Figure S2: Distant metastasis-free survival (A) and overall survival (B) in the Mayo SPORE post-prostatectomy cohort according to *HSD3B1* genotype.

Median follow-up in this cohort (4.9 years) was shorter than for the other two cohorts, and was particularly limited among the homozygous variant group (3.2 years). In view of the small number of events for DMFS (62) and OS (40) during the short observation period, the SPORE cohort was used to validate the impact of *HSD3B1* genotype on progression-free survival only (see text). Thus, the DMFS and OS curves above are presented for qualitative purposes only, as the data are not mature enough for reliable quantitative analysis of these endpoints. The above limitations notwithstanding, the homozygous variant group does appear to diverge.

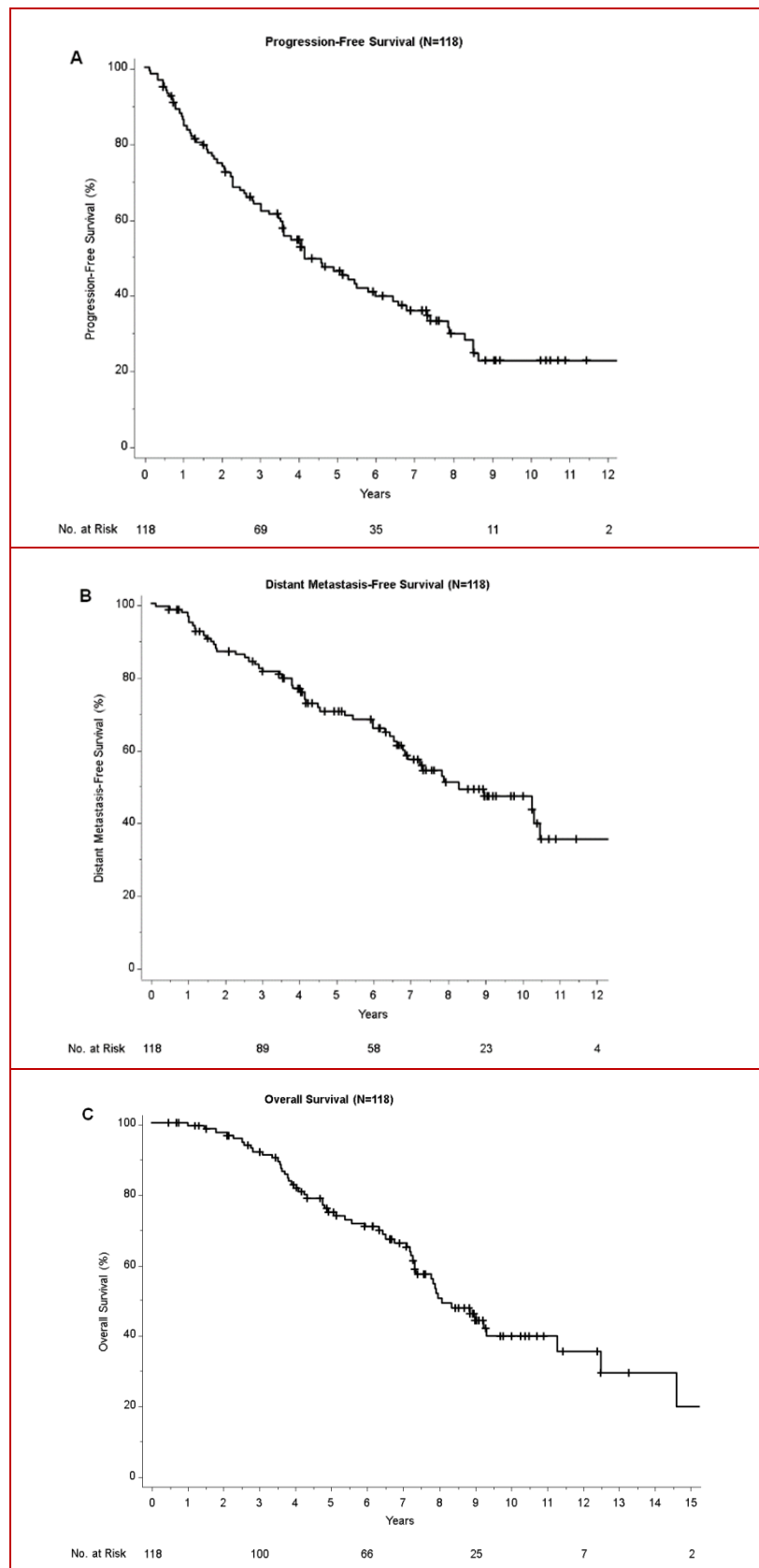


Figure S3: Progression-free survival (A), distant metastasis-free survival (B), and overall survival (C) in the primary study cohort (all genotypes combined).

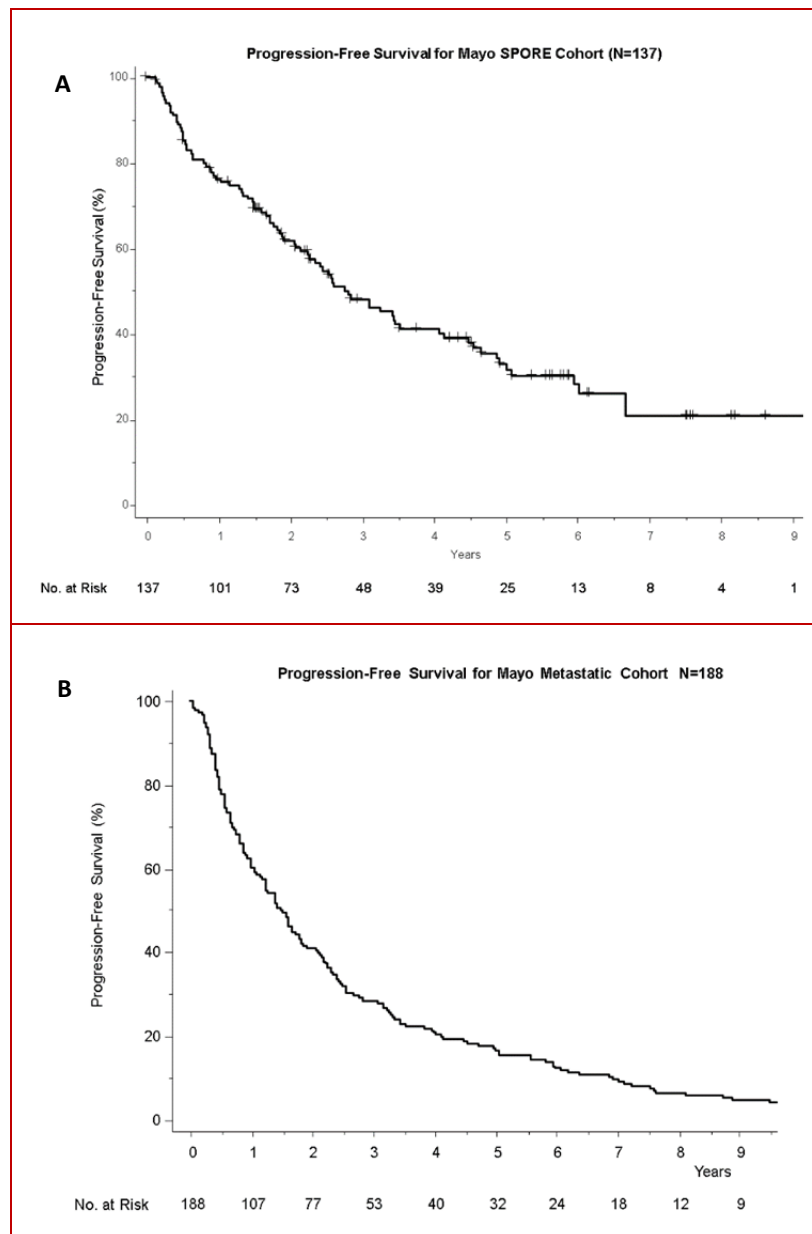


Figure S4. Progression-free survival in (A) post-prostatectomy and (B) metastatic validation cohorts (all genotypes combined).

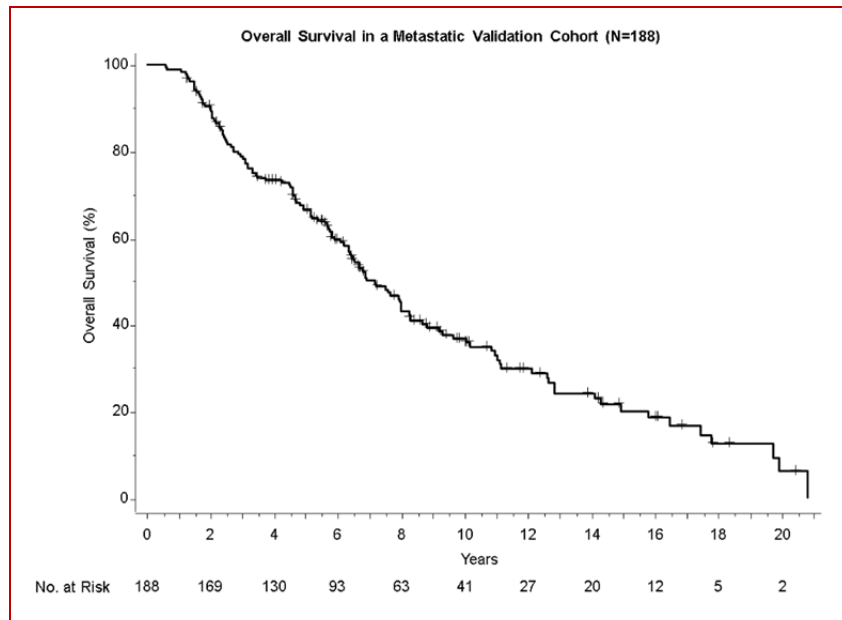


Figure S5: Overall survival in a metastatic validation cohort.

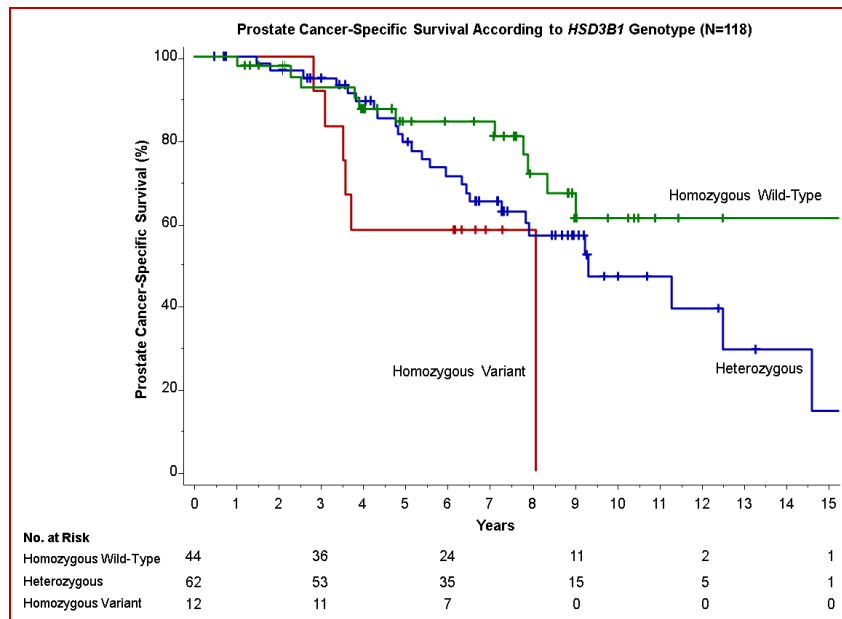


Figure S6: Prostate cancer-specific survival in the primary cohort according to *HSD3B1* genotype. Prostate-cancer specific survival diminished according to the number of variant *HSD3B1* alleles inherited ($P=0.029$).

Tables

Oligonucleotide	Sequence
Forward primer	5'-GTCAAATAGCGTATTACCTTCTCTTAT-3'
Reverse primer	5'-GAGGGTGGAGCTTGATGACATCT-3'
Unlabeled probe*	5'-GGAGA <u>A</u> CCTGAAGTCCAAGACTCAGTGATTTAAGG-3'
*The underlined bold character indicates the position of the mutation and the LNA base.	
Table S1: Oligonucleotide sequences used in this study	

Characteristic	Homozygous wild-type N = 77/137 (56%)	Heterozygous N = 50/137 (37%)	Homozygous variant N = 10/137 (7%)	P-value
Age (years)				0·47
Median	67	66	68	
Interquartile range	61-72	61-70	64-75	
Race – no. (%)				0·52
White	75 (97)	47 (94)	10 (100)	
Unknown	1 (1)	0	0	
Other	1 (1)	3 (6)	0	
T stage – no. (%)				0·20
T2	35 (46)	20 (40)	2 (20)	
T3a	8 (10)	12 (24)	1 (10)	
T3b4	24 (31)	10 (20)	2 (20)	
TX	10 (13)	8 (16)	5 (50)	
N stage – no. (%)				0·025
N0	67 (87)	42 (84)	5 (50)	
N1	10 (13)	8 (16)	5 (50)	
PSA at ADT initiation (ng/mL)				
Median	0·7	0·7	1·2	
Interquartile range	0·3-2·0	0·3-2·2	0·8-3·1	
Gleason score – no. (%)				0·99
≤6	5 (7)	4 (8)	0	
7	31 (40)	21 (42)	5 (50)	
8-10	37 (48)	23 (46)	5 (50)	
Unknown	4 (5)	2 (4)	0	
Neoadjuvant ADT – no. (%)				0·20
Y	19 (25)	6 (12)	2 (20)	
N	58 (75)	44 (88)	8(80)	
Adjuvant/Salvage RT – no. (%)				0·97
Y	31 (40)	29 (58)	6 (60)	
N	46 (60)	21 (42)	4 (40)	
<p>SPORE denotes the National Institutes of Health-funded Specialized Programs of Research Excellence. PSA denotes prostate-specific antigen level. Staging refers to the American Joint Commission on Cancer Tumor–Node–Metastasis (TNM) staging system. ADT denotes androgen deprivation therapy. P-values represent comparisons across the three genotypes.</p>				
Table S2: Demographic and treatment characteristics of the Mayo Clinic SPORE cohort.				

Characteristic	Homozygous wild-type N = 98/188 (52%)	Heterozygous N = 79/188 (42%)	Homozygous variant N = 11/188 (6%)	P-value
Age (years)				0·62
Median	65	67	65	
Interquartile range	60-70	60-73	57-68	
Race – no. (%)				0·74
White	94 (96)	76 (96)	11 (100)	
Unknown	1 (1)	2 (2·5)	0	
Other				
T stage – no. (%)				0·068
T1-2	43 (44)	28 (35)	1 (9)	
T3-4	45 (46)	39 (49)	6 (55)	
TX	10 (10)	12 (15)	4 (36)	
N stage – no. (%)				0·38
N0	41 (42)	32 (41)	3 (27)	
N1	27 (28)	21 (27)	6 (55)	
NX	30 (31)	26 (33)	2 (18)	
M stage at diagnosis – no (%)				0·43
M0	7 (7)	11 (14)	0	
M1	31 (32)	22 (28)	5 (46)	
MX	60 (61)	46 (58)	6 (55)	
PSA at ADT initiation (ng/mL)				0·21
Median	7·5	12·0	39·9	
Interquartile range	1·6-28·1	2·8-61·8	2·0-266·3	
Gleason score – no. (%)				0·42
≤6	9 (9)	6 (8)	0	
7	33 (34)	25 (32)	1 (9)	
8-10	52 (53)	41 (52)	9 (82)	
Unknown	4 (4)	7 (9)	1 (9)	
Chemotherapy – no. (%)				0·83
Y	56 (57)	48 (61)	6 (55)	
N	42 (43)	31 (39)	5 (46)	
PSA denotes prostate-specific antigen level. Staging refers to the American Joint Commission on Cancer Tumor–Node–Metastasis (TNM) staging system. ADT denotes androgen deprivation therapy. P-values represent comparisons across the three genotypes.				
Table S3: Demographic and treatment characteristics of the Mayo Clinic metastatic cohort.				

Characteristic	Progression-free survival		Distant metastasis-free survival		Overall survival	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
<i>HSD3B1</i> genotype						
Homozygous variant vs. homozygous WT	1.9 (0.8 to 4.2)	0.13	2.8 (1.1 to 6.7)	0.025	3.5 (1.3 to 9.5)	0.013
Heterozygous vs. homozygous WT	1.6 (0.9 to 2.7)	0.10	1.8 (1.0 to 3.3)	0.050	2.0 (1.0 to 3.9)	0.054
At least one variant allele vs. none*	1.6 (1.0 to 2.7)	0.074
T stage						
T3a vs. T2	2.5 (1.1 to 5.6)	0.033	1.1 (0.5 to 2.5)	0.74	1.0 (0.4 to 2.5)	0.94
T3b-4 vs. T2	2.5 (1.1 to 5.9)	0.030	1.1 (0.5 to 2.4)	0.77	1.5 (0.6 to 3.4)	0.40
T3b-4 vs. T3a	1.0 (0.6 to 1.9)	0.91	1.0 (0.5 to 1.9)	0.96	1.5 (0.7 to 3.1)	0.27
N stage						
N1 vs. N0 or NX	1.7 (1.0 to 3.0)	0.062	1.4 (0.8 to 2.5)	0.30	1.5 (0.8 to 2.7)	0.21
PSA at ADT initiation						
>2 ng/mL vs. ≤2 ng/mL	2.4 (1.5 to 4.0)	0.0003	2.2 (1.3 to 3.6)	0.0032	1.9 (1.1 to 3.4)	0.022
Gleason score						
8-10 vs. 6-7	1.3 (0.8 to 2.0)	0.36	1.8 (1.1 to 3.0)	0.023	1.7 (0.9 to 2.9)	0.076
HR denotes hazard ratio. CI denotes confidence interval. WT denotes <i>HSD3B1</i> homozygous wild-type genotype. PSA denotes prostate-specific antigen level. Staging refers to the American Joint Commission on Cancer Tumor–Node–Metastasis (TNM) staging system. ADT denotes androgen deprivation therapy. *When we evaluated the impact of inheriting at least one variant allele on progression-free survival, the adjusted outputs for covariates were nearly identical and thus the table was not duplicated (data not shown). .. = not assessed.						
Table S4: Multivariable analyses.						

Distribution of Progression Events

As mentioned in the text, progression-free survival was a composite endpoint. For the primary study cohort, the distribution of the component types of events was recorded. Of initial progression events, 70% were based on PSA criteria (second increase on ADT); many of these men subsequently progressed clinically (ultimately reflected in the distant-metastasis free survival and overall survival curves). The remaining 30% of initial progression events consisted of 22% that were based on clinical progression, and 8% based on initiation of second-line therapy.

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Association of *HSD3B1* Genotype With Response to Androgen-Deprivation Therapy for Biochemical Recurrence After Radiotherapy for Localized Prostate Cancer

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IMPORTANCE The variant *HSD3B1* (1245C) allele enhances dihydrotestosterone synthesis and predicts resistance to androgen-deprivation therapy (ADT) for biochemically recurrent prostate cancer after prostatectomy and for metastatic disease. Whether this is true after radiotherapy is unknown.

OBJECTIVE To determine whether the *HSD3B1* (1245C) allele predicts worse clinical outcomes from ADT for biochemical recurrence after radiotherapy.

DESIGN, SETTING, AND PARTICIPANTS The Prostate Clinical Research Information System at Dana-Farber Cancer Institute was used to identify the study cohort, which included men treated with ADT for biochemical recurrence after primary radiotherapy between 1996 and 2013. We retrospectively determined *HSD3B1* genotype.

MAIN OUTCOMES AND MEASURES Time to progression, time to metastasis, and overall survival according to genotype. Demographic and treatment characteristics were evaluated for confounders. Multivariable analyses were performed to adjust for known prognostic factors.

RESULTS A total of 218 eligible men were identified, of whom 213 (98%) were successfully genotyped. Of these, 97 of 213 (46%), 96 of 213 (45%) and 20 of 213 (9%) carried 0, 1, and 2 variant alleles. Overall variant allele frequency was 136 of 426 alleles (32%). Median patient age (interquartile range) was 69 (63-74), 72 (65-78), and 69 (65-77) years for 0, 1, and 2 variant alleles ($P = .03$). Demographic and treatment factors were otherwise similar. During a median follow-up of 7.9 years, median time to progression was 2.3 years (95% CI, 1.6-3.1 years) with 0 variant alleles, 2.3 years (95% CI, 1.5-3.3 years) with 1 variant allele, and 1.4 years (95% CI, 0.7-3.3 years) with 2 variant alleles ($P = .68$). Median time to metastasis diminished with the number of variant alleles inherited: 7.4 (95% CI, 6.7-9.7), 5.8 (95% CI, 4.9-6.5), and 4.4 (95% CI, 3.0-5.7) years, with inheritance of 0, 1, and 2 variant alleles, respectively ($P = .03$). Median OS was 7.7 (95% CI, 6.7-10.3), 6.9 (95% CI, 5.8-8.4), and 7.2 (95% CI, 3.8-7.9) years with inheritance of 0, 1, and 2 variant alleles, respectively ($P = .31$). On multivariable analysis with 0 variant alleles as the reference, the adjusted hazard ratio for metastasis was 1.19 (95% CI, 0.74-1.92) ($P = .48$) for 1 variant allele and 2.01 (95% CI, 1.02-3.97) ($P = .045$) for 2 variant alleles. Multivariable analysis did not demonstrate significant differences in TTP or OS.

CONCLUSIONS AND RELEVANCE In this study, the *HSD3B1* (1245C) allele was associated with more rapid development of metastases in men treated with ADT for biochemical recurrence after primary radiation therapy for prostate cancer. Notably, 105 of 213 men (49%) had received prior ADT, and 119 of 213 (56%) received an androgen receptor antagonist during salvage treatment, both of which may attenuate the effect of the variant allele.

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← Invited Commentary

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Since Huggins and Hodges¹ discovered the therapeutic impact of castration, androgen-deprivation therapy (ADT) has been the cornerstone of systemic therapy for prostate cancer and has improved overall survival (OS) in multiple contexts.²⁻⁵ While nearly all men will respond to ADT, most will eventually develop castration-resistant disease.^{6,7} There is wide variation in the duration of response to ADT, ranging from immediate progression to responses of several years.⁸ A germline variant, 1245A>C, in *HSD3B1* (OMIM 109715), encodes a more stable enzyme required for dihydrotestosterone synthesis from extragonadal precursor steroids.⁹ Inheritance of the variant *HSD3B1* (1245C) allele has been shown to predict intrinsic prostate cancer resistance to ADT in 2 postprostatectomy biochemically recurrent cohorts and a metastatic cohort.¹⁰ These findings were recently independently validated.¹¹ Whether the variant allele is also associated with inferior clinical outcomes for men treated with ADT in the context of biochemical recurrence after radiotherapy (RT) is unknown. We sought to investigate the impact of *HSD3B1* genotype in this setting.

Methods

We used the previously characterized Prostate Clinical Research Information System¹² at Dana-Farber Cancer Institute to identify men who were treated with ADT for biochemical recurrence after definitive RT. Key clinical data were extracted and systematically quality assured by 2 genitourinary oncologists. Biological specimens and clinical data were obtained in accordance with Dana-Farber institutional review board policy, and all patients provided written informed consent. The *HSD3B1* genotype was determined retrospectively from buffy coat using previously published methods¹⁰ by investigators blind to the clinical data.

Kaplan-Meier methods were used to assess time to progression (TTP), time to metastasis (TTM), and OS from initiation of salvage ADT. We analyzed differences in these end points according to genotype using an additive genetic model with the log-rank trend test. Progression was defined as the first occurrence of (1) prostate-specific antigen (PSA) progression; (2) radiographic progression; or (3) initiation of second-line therapy. We defined PSA progression as a minimum of 2 increases in PSA, with the date of first increase (nadir plus >0.02 ng/mL) as the progression date. For patients who received intermittent ADT, PSA increases during off-ADT periods were not counted as progression. Both TTP and TTM were censored on the date of last disease assessment for those who did not develop progression or metastasis.

Demographic and treatment characteristics were compared across genotypes to assess for confounders using Kruskal-Wallis and Fisher exact tests. Multivariable analyses were performed with Cox regression to adjust for known prognostic factors, including biopsy Gleason score, PSA at ADT initiation, age, and use of prior ADT with RT. $P < .05$ was interpreted as statistically significant. Analyses were performed with SAS software, version 9.4 (SAS Institute Inc).

Key Points

Question Does inheritance of the variant *HSD3B1* (1245C) allele predict worse clinical outcomes in men treated with androgen-deprivation therapy (ADT) for biochemically recurrent prostate cancer after primary radiotherapy?

Findings In this cohort of 213 men treated at a large academic center and retrospectively genotyped, inheritance of the *HSD3B1* (1245C) allele was not associated with a shorter time to progression or overall survival, but it was associated with shorter times to metastasis according to the number of variant alleles inherited.

Meaning The variant *HSD3B1* (1245C) allele was associated with more rapid development of metastases in men receiving ADT for biochemical recurrence after primary radiotherapy.

Results

We identified 218 men treated with ADT between 1996 and 2013 for biochemical recurrence after RT for localized prostate cancer, of whom 213 (98%) were successfully genotyped. Of these, 97 of 213 (46%), 96 of 213 (45%), and 20 of 213 (9%) carried 0, 1, and 2 variant alleles, respectively. The overall variant allele frequency was 136 of 426 alleles (32%). Median follow-up was 7.9 years. Demographic and treatment factors were similar across genotypes, although the median age at ADT initiation was slightly higher in heterozygotes than in the other 2 groups (Table).

Kaplan-Meier plots of time to event outcomes are shown in the Figure. Median TTP was 2.3 years (95% CI, 1.6-3.1 years) in men who inherited 0 variant alleles, 2.3 years (95% CI: 1.5-3.3 years) with 1 variant allele, and 1.4 years (95% CI: 0.7-3.3 years) with 2 variant alleles ($P = .68$). Median TTM diminished with the number of variant alleles inherited: 7.4 (95% CI, 6.7-9.7), 5.8 (95% CI, 4.9-6.5), and 4.4 (95% CI, 3.0-5.7) years, with inheritance of 0, 1, and 2 variant alleles, respectively ($P = .03$). Median OS was 7.7 (95% CI, 6.7-10.3), 6.9 (95% CI, 5.8-8.4), and 7.2 (95% CI, 3.8-7.9) years with inheritance of 0, 1, and 2 variant alleles, respectively ($P = .31$). On multivariable analysis with 0 variant alleles as the reference, the adjusted hazard ratio (HR) for metastasis was 1.19 (95% CI, 0.74-1.92) ($P = .48$) for 1 variant allele and 2.01 (95% CI, 1.02-3.97) ($P = .045$) for 2 variant alleles. Multivariable analysis did not demonstrate significant differences in TTP or OS.

Discussion

To our knowledge, this is the first study to analyze the association of *HSD3B1* genotype with response to ADT after RT for localized prostate cancer. Previous analyses in men treated with ADT for biochemical failure after prostatectomy or for metastatic disease have shown a strong association between *HSD3B1* genotype and response to ADT. In contrast to those studies, there was not a statistically significant association between genotype and composite TTP or OS in the current study. Nonetheless, we found a prominent difference in TTM, with differences between genotypes measured in years. These results are

Table. Demographic and Treatment Characteristics by *HSD3B1* Genotype^a

Characteristic	Patients, No.(%)			P Value ^b
	Homozygous Wild-Type (n = 97)	Heterozygous (n = 96)	Homozygous Variant (n = 20)	
Age at ADT initiation, median (IQR), y	69 (63-74)	72 (65-78)	69 (65-77)	.03
Clinical T stage at diagnosis				.73
T1	48 (50)	49 (51)	10 (50)	
T2	24 (25)	17 (18)	2 (10)	
T3-T4	4 (4)	3 (3)	1 (5)	
Tx	8 (8)	12 (13)	2 (10)	
Unknown	13 (13)	15 (16)	5 (25)	
Clinical N stage at diagnosis				.76
N0	37 (38)	38 (40)	7 (35)	
Nx	47 (48)	43 (45)	8 (40)	
Unknown	13 (13)	15 (16)	5 (25)	
Biopsy Gleason score				.43
≤6	29 (30)	20 (21)	2 (10)	
7	33 (34)	33 (34)	10 (50)	
8-10	28 (29)	32 (33)	6 (30)	
Unknown	7 (7)	11 (12)	2 (10)	
ADT as part of local therapy				.84
No	49 (51)	50 (52)	9 (45)	
Yes	48 (49)	46 (48)	11 (55)	
Year of ADT initiation				.66
1996-2000	25 (26)	25 (26)	8 (40)	
2001-2005	36 (37)	31 (32)	4 (20)	
2006-2010	31 (32)	32 (33)	6 (30)	
2011-2013	5 (5)	8 (8)	2 (10)	
ADT schedule				.93
Continuous	63 (65)	62 (65)	12 (60)	
Intermittent	34 (35)	34 (35)	8 (40)	
Received an antiandrogen during ADT				>.99
No	43 (44)	42 (44)	9 (45)	
Yes	54 (56)	54 (56)	11 (55)	
PSA at ADT, median (IQR), ng/mL	8.7 (4.6-18)	11.4 (6.4-19)	13.6 (6.0-27)	.13
Follow-up after ADT initiation, median (IQR), y	6.4 (4.1-10.9)	8.3 (4.1-13.0)	7.9 (5.9-13.6)	.56

Abbreviations: ADT, androgen-deprivation therapy; IQR, interquartile range; PSA, prostate-specific antigen.

^a Data are presented as number (percentage) of patients unless otherwise indicated.

^b Kruskal-Wallis test for continuous variables or Fisher exact test for categorical variables.

consistent with those published for the Cleveland Clinic post-prostatectomy cohort, in whom there was also a large difference in TTM according to genotype.¹⁰

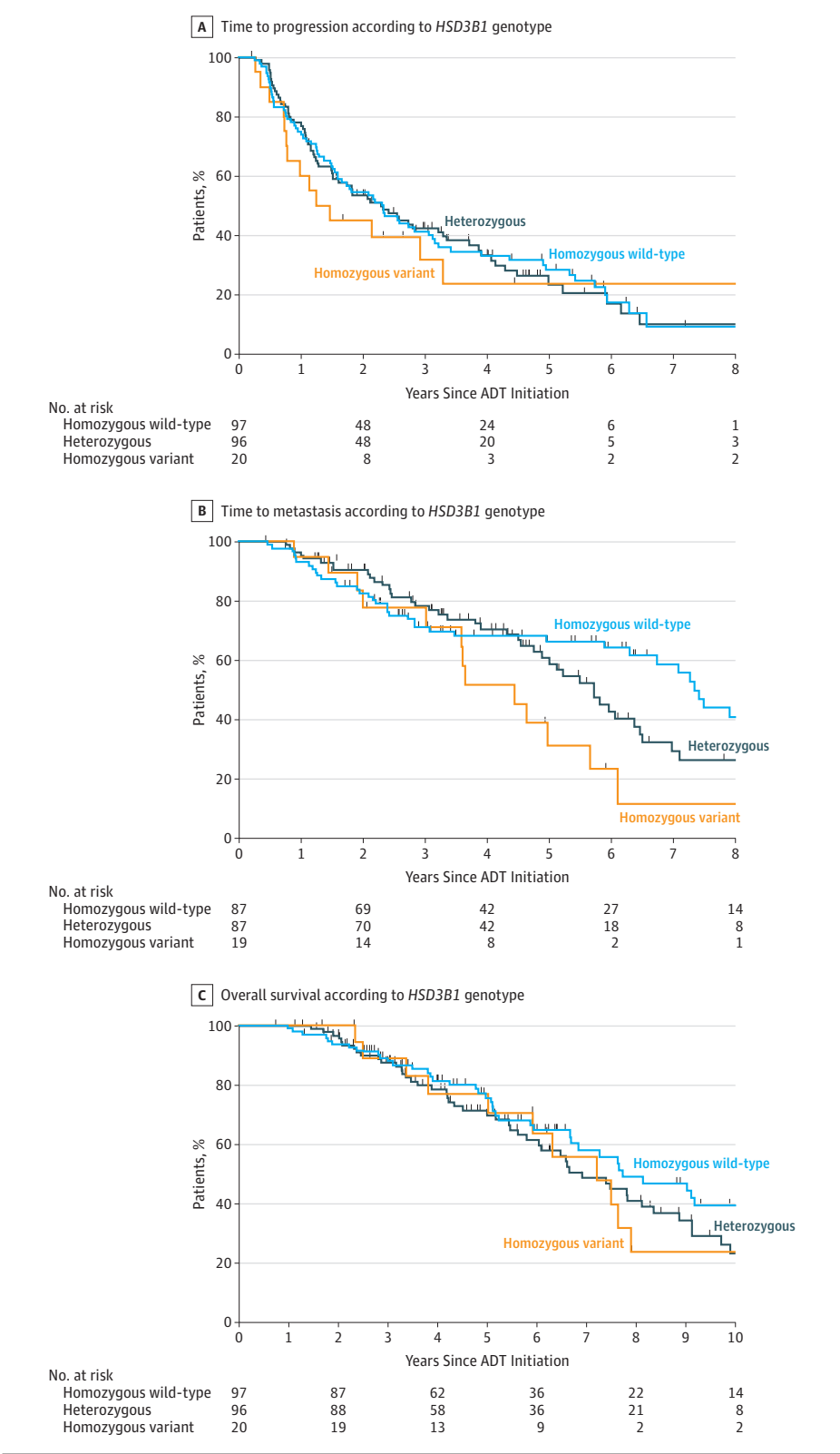
There are a few notable differences between our cohort and the previously published post-prostatectomy cohorts. In the present study 105 of 213 men (49%) had received prior ADT as part of RT. In contrast, few men had previously received ADT in the published cohorts. This is relevant, since the selective pressure from ADT ultimately leads to emergence of multiple somatic resistance mechanisms, which could dilute the impact of germline *HSD3B1* genotype. Moreover, 119 of 213 men (56%) in the present study received an androgen receptor (AR) antagonist during ADT, whereas the vast majority in the previously published cohorts did not. Although the *HSD3B1* (1245C) allele increases the rate at which adrenal androgen precursors are converted to dihydrotestosterone within tumor cells, AR an-

tagonists compete with intratumoral androgens and may attenuate the effect of the variant allele. Thus, it is possible that the high rates of prior ADT exposure and frequent use of AR antagonists during salvage ADT modified the impact of genotype with respect to composite TTP and OS. Nonetheless, the large impact on TTM is statistically and clinically significant. In addition, patients who receive definitive RT often differ from those who receive prostatectomy, and typically the former have more aggressive disease.¹³ Adverse genetics underlying more aggressive disease may thus be more prevalent in those treated with RT, which might also contribute to differences in the effect of *HSD3B1* genotype between cohorts.

Limitations

Regarding OS, a landmark trial of ADT for biochemical recurrence after RT demonstrated a median OS of 8.8 to 9.1 years,

Figure. Time to Progression (A), Time to Metastasis (B), and Overall Survival (C) According to *HSD3B1* Genotype



and this may be an underestimate with modern systemic therapy.¹⁴ Thus, the follow-up length for this cohort is inadequate to reliably exclude a difference in OS by genotype. Given

the degree of difference in TTM, one would expect that there may eventually also be a difference in survival, albeit delayed by several years. While speculative, it is noteworthy that the

OS curves for our cohort are potentially starting to diverge by approximately 7.5 years. Prostate cancer-specific mortality could not be analyzed, since cause of death data were not available. Finally, OS data may be confounded by subsequent therapies. As reported by Almassi et al¹⁵ in the companion article in this issue of *JAMA Oncology*, the variant allele has been associated with delayed progression in men receiving nonsteroidal CYP17A1 inhibition.

Conclusions

In conclusion, the *HSD3B1* (1245C) allele that enhances dihydrotestosterone synthesis from extragonadal precursor steroids is associated with more rapid development of metastases in men treated with ADT for biochemical recurrence after RT for prostate cancer.

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Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Hearn, Almassi, Sharifi.

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Conflict of Interest Disclosures: A patent application has been filed by Cleveland Clinic for a method of steroid-dependent disease treatment based on *HSD3B1*; Nima Sharifi, MD, is listed as co-inventor on this patent application. No other disclosures are reported.

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